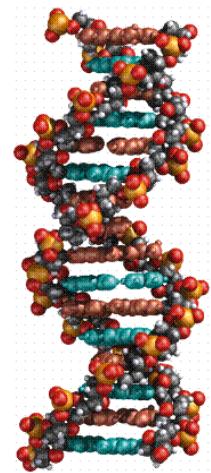


Principles of Toxicology



Course Notes
2013

for McGill University
Course OCCH-612



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[http://www.invitroplus.mcgill.ca/Ftp/Toxicology Course Notes 2013.pdf](http://www.invitroplus.mcgill.ca/Ftp/Toxicology%20Course%20Notes%202013.pdf)

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Determinants of Toxic Action

1 - REACHING VULNERABLE SITES

2 - TIME-COURSE OF DELIVERY

3 - DETOXIFICATION

4 - TOXICANT INTERACTIONS

5 - UNFOLDING OF TOXIC REACTIONS

Modeling Principles

1 - MODEL RELEVANCE

A - DESPECIATION

B - TOXICITY VARIABLES

C - TEST ENVIRONMENT

2 - LOW-DOSE EXTRAPOLATION

3 - LONG-TERM EXTRAPOLATION

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Key to Assignment 2: _____ Solution: _____

Key to Assignment 3: _____ Solution: _____

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2. Risk Assessment
3. Targets and Bio-Transformation
4. Toxicokinetics
5. Hemato- and Vascular Toxicity
6. Dermatotoxicity
7. Neurotoxicity
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1 · Scope of Toxicology

1.1. Toxicants



Toxicology studies the *injurious effects* of chemical and physical agents (including energy) on living organisms, observed as alterations in structure and function.

The variety of *injurious effects* becomes apparent if we examine the major causes of death (F1.1). Many of these diseases are

caused or accelerated by exposure to toxic substances.

Toxicity data from various bio-medical sciences document the effects of exposure to natural[▲] or artificial agents.

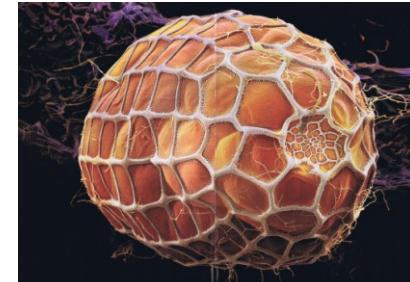
For more information on the history and scope of Toxicology, the student is referred to Casarett and Doull's *Toxicology. The Basic Science of Poisons*. 6th Edition, Curtis Klaassen, McGraw Hill, 2001, pp. 3-10.

[▲] to dispel the idea that *natural is by definition healthy*, consider the following.

- many medicinal plants used by nearly 100 cultures on different continents are related, testifying to their efficacy. In excess, such natural medicines can involve risk.
- The hemlock plant (at right) was the state method of execution in ancient Greece, Socrates was one of its victims.
- Honey bees gather pollen from a variety of natural flowers which may contain toxins. From rhododendrons, one can get grayanotoxins, which bind reversibly to cell membranes, allowing the passage of sodium and leading to weakness, slow heartbeat, perspiration and nausea (the symptoms of a heart attack).
- Broccoli, considered a health food, contains methyl bromide, a commercial fumigant and pesticide. When crushed, its tissue generates isothiocyanates (toxic).
- The zebra longwing butterfly gathers toxicants ingested from plants they eat, and produce eggs laced with cyanide and other toxins (the egg is illustrated below).



Hemlock (*Conium maculatum*)



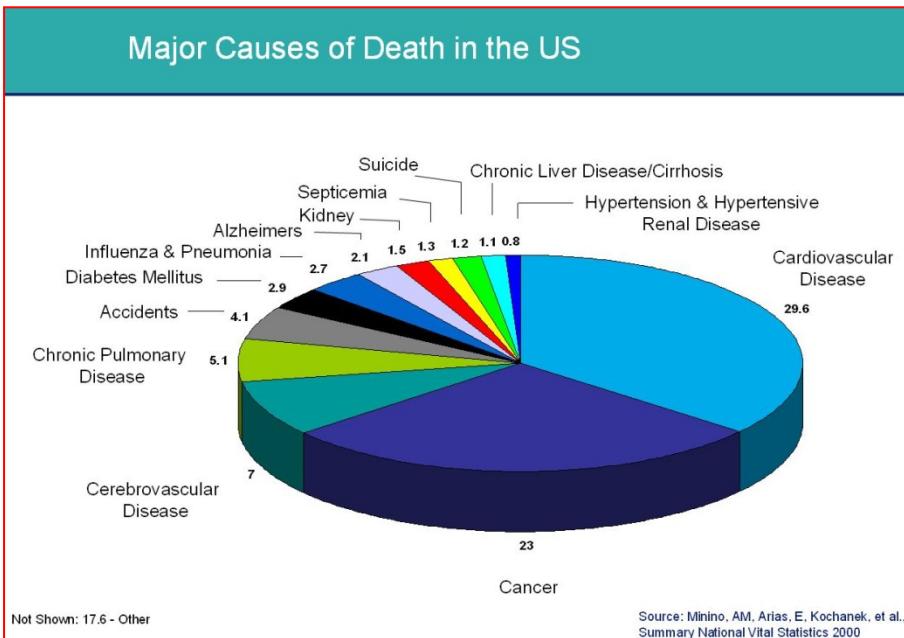
Egg of the zebra longwing butterfly

Toxicant Control

For pharmaceuticals and pesticides, producers have the burden of demonstrating safety to government. By contrast, for industrial chemicals, government must prove that a chemical poses a risk before it can restrict its production or use. But companies have little incentive to develop information, since this increases the likelihood that evidence of harm will be uncovered. Changes are on the way, however:

- Canada recently completed the Domestic Substances List (DSL), mandated by law in 1999, which for the first time examined information available on the roughly 23,000 previously unassessed chemicals that have been in commerce in Canada over the last two decades, and identified more than 4,300 warranting further scrutiny.
- In the U.S., the voluntary High Production Volume (HPV) Chemical Challenge compiles basic hazard information on some 2,000 of the highest-volume chemicals in use.
- In Europe, a system of Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) was adopted in December 2006. It requires producers and users of 30,000 chemicals in commerce in Europe to register them and to provide information on their production, use, hazard and exposure potential. Chemicals of very high concern will be allowed only by authorization.

Dealing with natural toxicants, such as plant extracts, is different from dealing with a purified chemical. When preparing potions based on plant sources, one has to take into account the fact that the initial material may contain more or less of the active agent, depending on season, growth conditions or even time of day. This is why the pharmaceutical industry generally looks for natural remedies to provide *leads*



F1.1. Causes of Death involve many diverse diseases and body systems.

to useful molecules, as opposed to using the grown material itself. Scientists in laboratories are equally concerned with conducting studies using well defined agents. The supply of uniform products is better served by synthesis laboratories than agriculture. This has the consequence that most prepared drugs are based on a few purified molecules, as opposed to complex mixtures. This purity may have undesirable consequences, in

particular by challenging metabolic pathways in very specific and forceful ways.

Almost any substance can be a toxicant. A single atomic species, such as metal exposure in industry (welding fumes), can have toxic effects. A protein, such as the *Clostridium Botulinum* toxin (F1.2), although much more complex, also has very strong toxic properties.

Subtle Toxicity

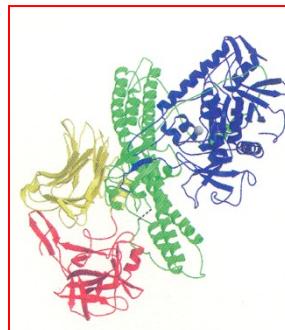
- + In the liquid state, the O-H distance is 3 % shorter in deuterium than in normal water, and heavy water freezes at 4°C, rather than 0°C. Although these differences appear small, they are enough to underlie large toxicity differences. Heavy water interferes with the mitotic apparatus of higher organisms, including mammals, who soon become ill and die at the point where about half their body water has been replaced. Bacteria are only able to grow slowly in pure heavy water.
- + Ionic pores are highly specific to metallic ions, but the selectivity of the pores is insufficient to avoid the substitution of lead for calcium, because both have similar atomic size (180 pm, see F7.4). This is also true for strontium, which has a slightly different size (200 pm), but a very similar electronic structure. Small differences in the properties of atoms have huge consequences for toxicity.
- + Sometimes, the same molecule can have both a toxic and a benign character. Hemoglobin started its career in worms as a binding agent to detoxify oxygen. It was ultimately adopted by mammals in a slightly altered form for oxygen transport. Even within mammals, it transports life-giving oxygen to tissues, but will destroy the kidney's glomerulus, if liberated unchecked into the blood.

It seems, from these examples, as if toxicity occurs *by accident*.

Not-so-Subtle Toxicity

By contrast, some toxic molecules seem entirely conceived for nefarious goals. In the Botulinum toxin molecule of F1.2, *Blue* is the toxic fragment which paralyzes the muscles, *Green* is the pore opening the muscle's membrane, *Red-Yellow* binds to receptors on nerve cells, and if you look closely, a green loop is designed to mask the toxic part (*Blue*) from the immune system.

F1.2. Molecular Structure of the *Botulinum* toxin. Made of 1285 amino acids, 100 billionth of a gram is lethal through interference with acetylcholine.



As well, some substances are extremely hazardous when released accidentally as a result of chemical spills, industrial explosions, fires, or accidents involving railroad cars and trucks, or as a result of improper storage. These chemicals have been studied specifically to design emergency responses following releases.

Treating Acute Toxicity

Airway-breathing and circulation are always the most urgent considerations in emergency situations, to correct hypoxia and acidosis, and to maintain adequate circulation.

Documenting the intoxication is paramount.

Identification of electrolyte imbalances (renal function) and liver impairment should be assessed using basic laboratory studies (metabolic profile). Unless a specific antidote is available, management is supportive (treating symptoms) in

many cases. Mental or hemodynamic status may deteriorate rapidly. Children are particularly susceptible.

Single-dose activated charcoal is the preferred gastrointestinal tract decontamination, up to 1 hour after ingestion.

The severity of symptoms and toxicokinetics of the ingested substance are considered in the disposition of an intoxicated person. Observation time is based on toxicity, half-life and amount ingested, particularly if signs or symptoms of toxicity do not reverse during the observation period.

Making Toxicity

The human body itself generates toxins¹⁹, and may transform molecules into more dangerous species by the process of bio-activation (see Chapter 8). Further, molecules in the body can become toxins as a result of changes in folding conformation, without any covalent chemical change. Such changes lead protein to form aggregates, or to be misrouted within cells.

Examples of aggregation problems are found in diseases such as Alzheimer's, Creutzfeldt-Jakob and adult-onset diabetes for protein¹, and in Rett syndrome for chromatin²⁰. Examples of misrouting are cystic fibrosis, systemic amyloidosis, nephrogenic diabetes insipidus, cancer, and so on.

Pairs of molecules that are mirror images of each other can have lethal limits that are 17-38 times that of the other (pyrethroid insecticides).

Fighting Toxicity

Our fight against toxicants is an on-going story. Analysis of samples collected in 2001 and 2002 by the CDC in the US shows that within 2 years, hygiene effectively reduced body burdens of lead (in children, a decline from 2.2 to 1.7 µg/dl),

¹⁹ A *toxin* is a metabolically produced toxicant. A *toxoid* is an altered form of a toxin, possessing little or no toxic power, but capable of inducing antibodies.

second-hand smoke (blood cotinine declined by 70 %) and mercury (an 18 % decline to 0.83 µg/dl for women of childbearing age), while other agents such as benzo(a)pyrene (found in a quarter of urine samples) and diisobutyl phthalate (majority of people) are found to be more widespread than expected²⁶.

1.1.1. Identifying Intoxication

Many intoxications cause common diseases and diffuse, non-specific symptoms. Exposure history is vital for correct diagnosis, but primary care physicians include mention of the patient's occupation in only 24 % of cases, and only 2 % include information about (1) toxic exposure, (2) work history and (3) environmental history.

1.1.1.1. Toxic Exposure

Current and past exposures to chemicals (air pollution, pesticide exposure, hazardous wastes/spill exposure, metals), dust and fibers, fumes, biological hazards, radiation, noise, and/or vibration should be documented. “Are family members experiencing the same, or unusual symptoms?”

1.1.1.2. Time-Course of Intoxication/Symptoms

The time-course of symptoms is a major clue to uncovering intoxication. Malaise can be periodic if the exposure is rhythmic, as occurring during a work shift.

“Do your symptoms get either worse or better at work?” and “Do your symptoms get either worse or better on weekends?” In chronic intoxications, loss of appetite and weight are common symptoms.

The muscle inhibition of curare is more obvious than the nervous system hyperactivity connected with glutamate. Generally, the slower the intoxication, the more difficult it is to relate to a specific situation.

In liver cirrhosis, alcohol can cause infiltrations in liver tissue over long periods of time. Nephrotoxic agents can induce very slow loss of nephrons in the kidney.

1.1.1.3. Work History

- Description of all previous jobs including short-term, seasonal, and part-time employment and military service.
 - Description of typical workday (job tasks, location, materials, and agents used) and changes in routines or processes.
 - Jobs of household members.
- “Are other employees or family members similarly affected?”

1.1.1.4. Environment History

- Present and previous home locations, recent renovation/remodeling.
- Home ventilation/moisture control, insulation, heating and cooling systems.
- Home cleaning agents.
- Water supply.
- Hobbies (e.g., painting, photography, sculpting, welding, woodworking, piloting, restoring automobiles, shooting firearms, creating stained glass, creating ceramics, and gardening).

1.1.1.5. Forensics

To “home in” on a toxicant in the human body, blood can be used to indicate body status at a given time, but biopsies from heart, liver, kidney, lungs, brain, spleen, hair and nails are very useful to gauge chronic intoxication.

Unless you know what to look for, a large number of tests may be needed to finally detect a poison. But in acute cyanide poisoning, the odor is usually quite apparent at autopsy. You may perceive the bitter almond taste of cyanide if you crush 2 or 3 apple seeds between your teeth.

1.1.2. Identifying Toxicants

It is often difficult to distinguish cause from effect in biology, and this is particularly true in chronic toxicology. Symptoms can be connected to organs, which can be connected to histological changes, which may be connected to specific agents.

An important problem is that the reaction of the body to a toxicant may increase as a function of time because of bio-accumulation, or may attenuate because of physiological adaptation (toxicodynamics).

1.1.2.1. Structure-Activity Relationships

The ability to predict from molecular structure alone which molecules are toxic in the human body would be extremely precious. Unfortunately, our knowledge is not so advanced. Generally, real hints on toxicity purely from chemical structure are slim. In the absence of test data, Structure-Activity Relationship (SAR) analysis, which tries to predict a chemical's toxicity based on its chemical structure, is of limited usefulness. Accuracy of SAR analysis varies considerably depending on the particular chemical and on the predicted characteristic. Unless some toxicity information is already available on a specific molecule or on a closely related molecular structure, we depend on experiments of various kinds.

1.1.2.2. Toxicants and Evolution

In evolutionary development, tremendous efforts have been made to help animals identify toxicants. The largest gene superfamily in vertebrates is that of the olfactory receptor genes. In the mouse, 1,296 olfactory genes in 27 clusters have already been identified¹³.

1.1.2.3. Reaching the Target

In the case of drugs, identification has traditionally been largely a matter of trial and error. A similar problem exists in the process of drug discovery, where a number of laboratories race to find molecules with specific medicinal actions.

The *Lipinsky¹¹ rules* predicting which molecules make good drugs (to be taken by mouth) relate essentially to *absorption* of the chemical, as opposed to its physiological action: molecules that cannot reach their targets cannot be effective drugs.

T1.3. LIPINSKY RULES FOR DRUGS

- | |
|---|
| 1. Molecular weight less than 500. |
| 2. Log of the Partition Coefficient less than 5. |
| 3. Number of hydrogen bonds donors less than 5
(expressed as the sum of OHs and NHs). |
| 4. Number of hydrogen bond acceptors less than 10
(expressed as the sum of Ns and Os). |
| 5. No more than 5 fused rings. |

- ✚ Large molecules generally have poor cell membrane penetration (#1 and #5 above).
- ✚ The *Partition Coefficient** of the molecule should not be too high, otherwise the molecule will not be able to cross fatty cell membranes *and* travel through body fluids (#2 above).
- ✚ Hydrogen bond donors and acceptors are known to impair crossing of cell membranes (#3, #4 above).

* The Partition Coefficient is a ratio of the equilibrium concentrations of a dissolved substance in a two-phase system made of two immiscible solvents (water and n-octanol).

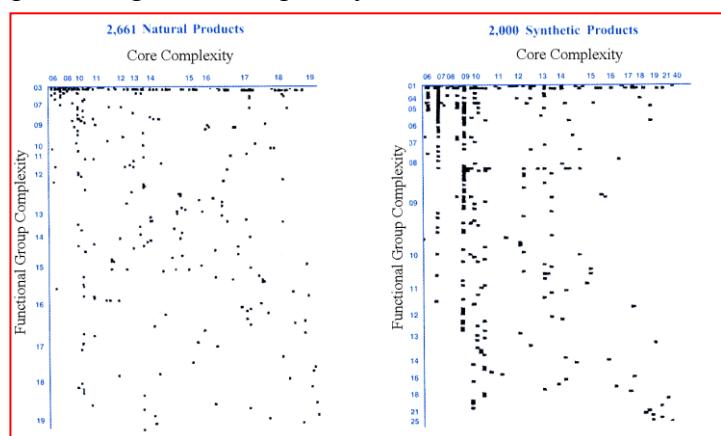
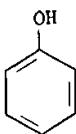
1.1.2.4. Scatter-graph of Drugs

So, is it entirely impossible to predict biological action of a chemical? One way to find out is to look at the chemical properties of a group of chemicals that all have some action on the body: drugs.

A basic way of describing drugs distinguishes *molecular core* from *functional groups*.

- + *Core complexity* counts all possible ring sizes, from largest to smallest. This is a measure of the complexity of the molecules' basic core.
- + *Functional group complexity* compiles all the possible ways of counting functional groups, from longest to shortest. This is a measure of the complexity of the molecules' functional groups.

In phenol, the *core* is benzene and the *functional group* is OH. If one draws diagrams (F1.4) positioning thousands of natural and synthetic drugs using axes representing *Core complexity*



F1.4. Scatter diagrams for natural and synthetic drugs.

and *Functional group complexity*, one does not see any groupings (F1.4 left).

The random scatter in natural products is thought to reflect unpredictability of physiological action, while the vertical concentrations in the synthetic products (F1.4 right) are thought to represent the practical preferences of synthesis chemists¹².

1.1.2.5. Antibiotics

The pharmaceutical industry has invested large efforts into the design of antibiotics (bacterial toxicants). Rather than focusing on specific structural chemical species, the strategy has generally been to address 4 major targets rather specific to bacteria:

- + the cell wall, a structure found in bacteria, but not animals (β -lactam rings),
- + protein production,
- + DNA and RNA replication (bacteria divide very rapidly), and
- + folate synthesis, a nutrient which donates one carbon unit in various biosynthetic pathways.

In the future, as a result of the development of molecular biology, drug identification may depend more on knowledge of genes, proteins and biochemical pathways.

1.1.3. Classifications of Toxicants

1.1.3.1. Classification by Function

Toxicants that have similar effects or uses can be grouped together, often reflecting applications. Frequently, compounds that are chemically related are used for similar purposes and form sub-groups within the general classifications.

Since bacteria are increasingly evading even the most potent front-line drugs, as resistance continues to rise, there is a clear need for new antibiotics. But the production of new drugs has slowed to a crawl since the 1940s and 1950s. In the last 50 years, just one antibiotic of an entirely new class has made it into clinical practice.

Looking for new antibiotics, some scientists turned to frog peptides, because most of our infections occur across mucus membranes. Because a frog's skin is essentially a moist mucus membrane, it presumably need something to protect it against pathogens. But despite decades of work, and thousands of candidate compounds, not a single amphibian antimicrobial peptides has turned into a marketable drug.

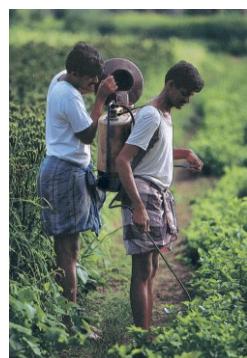
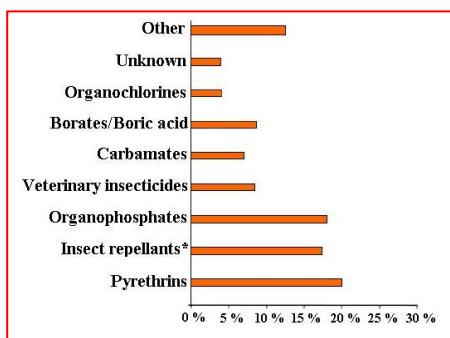
The compound that came closest to success (magainin), derived from the skin of the African clawed frog—passed through Phase II clinical trials for patients with diabetic foot ulcers, but the drug failed to get approval from the US FDA in 1999. Large peptides make poor drugs: some get metabolized too readily, while others trigger immune reactions. Their size also makes it difficult to synthesize them in large enough quantities.

1.1.3.1.1. Pesticides

Insecticides

F1.5. Application of parathion insecticide in India.

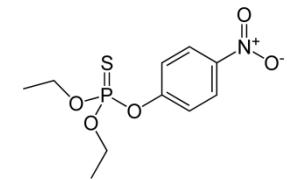
Most insecticides disrupt the nervous system.



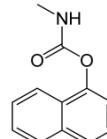
F1.6. Use of insecticides-pesticides in the US (1997-2000).

Under the same doses by weight as insects, humans show similar symptoms.

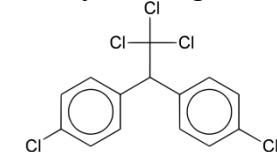
Organophosphates: parathion (shown, a pale yellow liquid), diazinon, malathion[¶]. Inhibit cholinesterase. Peripheral and CNS toxicity. Degraded by the liver. Not usually persistent in the environment.



Carbamates: aldicarb, carbaryl (shown), propoxur. Inhibit cholinesterase. Peripheral and CNS toxicity. Rapidly degraded.

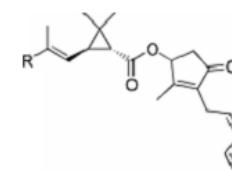


Organochlorines: dichlorodiphenyltrichloroethane (DDT, shown). Change cell membrane ion permeability, leading to altered firing rate. Readily stored in fat.



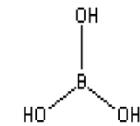
Metabolized at a rate of 1 % per day. Bio-accumulates in the food chain. Banned in 1972 in the US.

Pyrethrins: synthetic versions of the pesticide pyrethrin occurring in the flower chrysanthemum. Modified to increase their stability in the environment. Some synthetic pyrethroids are toxic to the nervous system.



Pyrethrin I = R-CH₃ Pyrethrin II = R-CO₂-CH₃

Boric acid: (borax and boron-containing salts): low-toxicity minerals with insecticidal, fungicidal, and herbicidal properties. Do not evaporate or volatilize into the air or pose the same health concerns as synthetic pesticides. Slower acting than the synthetic pesticides like diazinon or pyrethrins, but highly effective over a long period of time. As an insecticide, boric acid acts as a stomach poison for ants, cockroaches, silverfish and termites,



[¶] Malathion is being phased out for many uses in Quebec in 2005. Methyl parathion is now banned for use on most fruits and vegetables in the US because of effects on the development of children's nervous system (see Chap. 7). It is used in lice shampoos.

and as abrasive to the insect exoskeleton. As an herbicide, boric acid causes desiccation or interrupts photosynthesis in plants. Ingestion of boric acid leads to a dermal rash called “boiled lobster syndrome”.

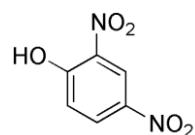
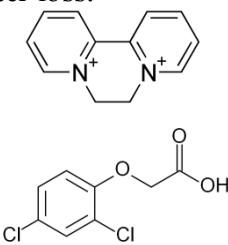
Herbicides

Mostly of low human toxicity, because of physiological differences between humans and plants. Interfere with plant hormones, inhibit photosynthesis, or promote water loss.

Bipyridyls: diquat (shown), paraquat. Desiccate plants. Ingestion of concentrated paraquat almost always leads to death due to lung failure (see Chapter 11).

Chlorophenoxy compounds: 2,4-D^Σ (shown) and 2,4,5-T. Agent Orange, a defoliant used in the Vietnam war, was a 50-50 mixture of above compounds. Promote uncontrolled growth, leading to rapid plant death. Weakly toxic to humans, but a trace impurity of 2,4,5-T is dioxin (a teratogenic, carcinogenic compound causing chloracne).

Dinitrophenol: 2,4 dinitrophenol (DNP). Inhibits ATP synthesis. Marketed as an anti-obesity agent between 1935 and 1937.



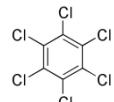
Fungicides

Hexachlorobenzene (shown) and Organomercurials:

Are no longer marketed, but were used to treat seed grain.

In various incidents, *seed* was mistaken for *grain* in Turkey and Iraq, and was consumed by humans.

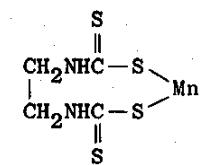
Phthalimides: use halted. Structure similar to thalidomide.



^Σ Quebec is banning 2,4-D on the basis of possible carcinogenicity and endocrine and nervous system disturbances.

Dithiocarbamates: maneb (shown), zineb, mancozeb.

These three fungicides are metallo co-ordination complexes with ethylenebis (dithiocarbamate), and exist as polymers. The manganese and zinc coordination complexes are known as maneb and zineb, respectively. In use, also used as insecticide.

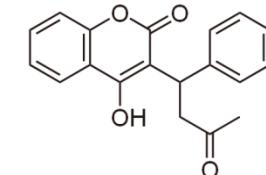


Rodenticides

Most exposures are from accidents in industry, since they are directly applied to palatable animal baits.

Anticoagulants: warfarin (shown).

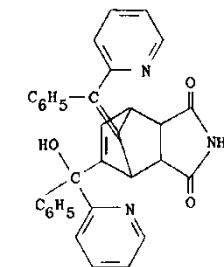
Inhibits synthesis of vitamin K, leading to elimination of prothrombin and absence of clot formation. Animals bleed to death.



Inhibitors of cellular respiration:

derivatives of fluoroacetic acid. Block many enzymes in the Krebs cycle, impeding ATP production.

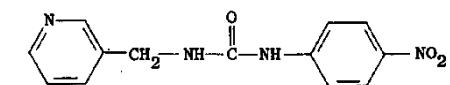
Vasoconstrictors: norbormide (shown). Triggers a vascular reaction *specific to rodents*, leading to necrosis and death. Similar responses are not seen in humans with doses 30 times larger.



Diabetogenics: pyriminil (shown). Blocks nicotinamide metabolism, leading to death from paralysis and respiratory arrest. Supposed to be non-toxic to humans, but is

noted to destroy beta-cells of the pancreas, leading to a diabetic state. Peripheral

neuropathy and numerous Central Nervous System effects have been noted. Antidote is nicotinamide.



1.1.3.1.2. Metals

Many metals have variable oxidation numbers, which presents an opportunity for physiological use (ex, transport of oxygen by the iron of hemoglobin), but also for toxicity. They may

substitute one another (ex, lead for calcium) in terms of chemistry or size (at cell membrane pores), but carry subtly different characteristics that lead to toxicity. For example, lead has a broader chemistry than calcium. Calcium prefers oxygen ligands, while lead will also complex with the sulphhydryl group, and form complex ions with OH^- , Cl^- , NO_3^- and CO_3^{2-} . Metals also interfere in neurotransmission and energy metabolism, stimulate reactions, interact with DNA and create free radicals.



Fig. 1.6a. Worker mining salt in Lake Katwe, Uganda. Any skin injury will allow salt, Cu, Pb, Cd and Zn to enter the body, preventing healing.

Metals may present in elemental, inorganic or organic forms.

- ⊕ Some atomic forms of metals (in red in T1.7) are outright toxicants, having no useful metabolic function:
Be, Ga, Cd, Hg, Tl, Pb, Bi, Ra, U.
- ⊕ Some metals (in green in T1.7) are essential to humans:
Na, Mg, K, Ca, Fe, Cu, Zn, Mo.
- ⊕ Others (in yellow-green in T1.7) are also essential, but are toxic at high doses: V, Cr, Mn, Co, Ni.
- ⊕ Still others (●) have important long-term toxicity:
Be, Cr, Mn, Ni, Cd, Hg, Pb.
- ⊕ While others (○) are carcinogenic in animal tests:
Be, Cr, Co, Ni, Cd, Sb, Pb.

Molybdenum (atomic number 42) is the only second-row transition metal that is required by most living organisms, and the few species that do not require molybdenum use tungsten (W), which lies immediately below molybdenum in the periodic table¹⁰.

T1.7. TOXICITY OF METALS displayed on the PERIODIC TABLE OF THE ELEMENTS																		
1 H																	2 He	
3 Li	4 Be ●●												5 B	6 C	7 N	8 O	9 F	10 Ne
11 Na	12 Mg												13 Al	14 Si	15 P	16 S	17 Cl	18 Ar
19 K	20 Ca	21 Sc	22 Ti	23 V	24 Cr ●●	25 Mn ●●	26 Fe	27 Co ●●	28 Ni ●●	29 Cu	30 Zn	31 Ga ●●	32 Ge	33 As	34 Se	35 Br	36 Kr	
37 Rb	38 Sr	39 Y	40 Zr	41 Nb	42 Mo	43 Tc	44 Ru	45 Rh	46 Pd	47 Ag	48 Cd ●●	49 In	50 Sn	51 Sb ●●	52 Te	53 I	54 Xe	
55 Cs	56 Ba	57 La *	72 Hf	73 Ta	74 W	75 Re	76 Os	77 Ir	78 Pt	79 Au	80 Hg ●●	81 Tl	82 Pb ●●	83 Bi ●●	84 Po	85 At	86 Rn	
87 Fr	88 Ra	89 Ac	104 Rf	105 Db	106 Sg	107 Bh	108 Hs	109 Mt									71 Lu	
					58 Ce	59 Pr	60 Nd	61 Pm	62 Sm	63 Eu	64 Gd	65 Tb	66 Dy	67 Ho	68 Er	69 Tm	70 Yb	
					90 Th	91 Pa	92 U ●●	93 Np	94 Pu	95 Am	96 Cm	97 Bk	98 Cf	99 Es	100 Fm	101 Md	102 No	
																	103 Lr	

January 22, 2013: after 4 years of negotiations, more than 140 nations signed the Minamata Convention, a treaty to reduce global mercury emissions by 2020. But limits on the use of mercury in coal power plants—one of the two main sources—won't go into effect for 5 to 10 years. Mercury is thought to impact the productivity of populations by reducing their ability to learn.

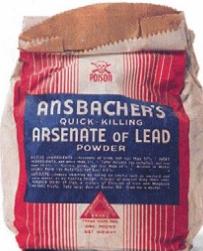
In the 1600s, women in Europe used white lead face paint, and mercury sulphide for rouge. The white lead made their hair fall out, which explains the fashion for high foreheads, as hairlines receded. Lead carbonate was used as a contraceptive in Rome (topical application).

Bacteria will grow over pieces of solid lead. Lead “resistance” in bacteria has been attributed to efflux pumps, P-type ATPases that are known to also transport Pb^{2+} , Cd^{2+} and Zn^{2+} across cell membranes¹⁹.

These ATPases are present in bacteria and mammalian membranes, and handle cations such as calcium, sodium-potassium and protons.

In the Middle Ages, syphilis was treated with mercury, which was toxic to the bacterium (the spirochete *Treponema pallidum*), but also to patients.

Later, potassium iodide was used. In the early 1900s, arsenic compounds ($C_{12}H_{12}N_2As_2O_2$) were introduced*. But occasionally, the liver would break down the organic arsenic compound into elemental arsenic, which is toxic to the liver. A protective measure to avoid this toxicity was to give patients injections of vitamin C.



Criminals favor arsenic because it is tasteless and odorless. Arsenic exploits cellular pathways and binds to critical proteins. Chronic administration produces weakness, confusion and paralysis. Acute ingestion (a few mg/kg) induces nausea, vomiting, diarrhea, low blood pressure and death.

In the early 1940s, penicillin replaced arsenic as the treatment of choice for syphilis. Arsenic derivatives are still used to treat African sleeping sickness and remains an effective chemotherapy for acute forms of leukemia.

Metals are often eliminated through the kidneys, which become targets themselves. Many metals are suspected of carcinogenicity, although we do not understand the exact mechanisms.

* Inorganic arsenic is a carcinogen (arsenite ions probably cause extra blood vessels to develop around tumors), while organic forms are less toxic, combat animal diseases and accelerate growth.

T1.8. Essential Metals.		
Metal	Function	Excess Produces
Cr	A common ingredient in vitamin and mineral supplements. Formation of glucose tolerance factor and for many enzyme reactions.	Hexavalent chromium (+ 6) is a carcinogen (inhaled form).
Co	In vitamin B-12	Polycythemia, Cardiomyopathy.
Cu	Synthesis of hemoglobin	Microcytic Anemia.
Fe	Erythropoiesis (formation of RBCs)	Liver and Cardiovascular damage. If inhaled, Silicosis-like symptoms.
Mn	Mn Superoxide Dismutase is an important protector of mitochondria.	Manganese Pneumonitis, CNS disorders.
Mo	Aldehyde oxidase, Sulfite oxidase and Xanthine oxidase.	Anemia and Diarrhea (animal studies).
Se	Glutathione peroxidases neutralize hydrogen peroxide from metabolism.	Neuropathies, Dermatopathies, Decreased Fertility, Teratogenesis.

1.1.3.1.3. Organic Solvents

Organic solvents are widely used in industry and fall into a number of classes.

Aliphatic Hydrocarbons: methane, ethane, propane, butane, pentane, hexane, heptane, octane, ethylene, propylene, butadiene, and isoprene.

Aromatic Hydrocarbons: benzene, toluene, styrene, ethylbenzene, xylene and naphthalene.

Halogenated Hydrocarbons: methyl chloride, methylene chloride, ethyl chloride, chloroform, methyl chloroform, bromoform, carbon tetrachloride and vinyl chloride.

Esters: acetate, ethyl silicate, ethyl formate, and methyl formate.

Ketones: methyl ethyl ketone, methyl isobutyl ketone, and diisobutyl ketone.

Ethers: methyl ether, isopropyl ether, chloromethyl ether, and chloromethyl methyl ether.

Alcohols: methanol, ethanol, propanol, isopropanol, isobutanol, amyl[↓] alcohols, and allyl[♦] alcohols.

Aldehydes: acrolein, acetaldehyde, formaldehyde, furfural, and chloral hydrate.

Amines: methylamine, dimethylamine, trimethylamine, ethylamine, diethylamine, triethylamine, propylamine, butylamine, allylamine and cyclohexylamine.

Organic solvents, because of their solubility in cell membranes, often depress the Central Nervous System and irritate tissues and membranes. Many are anesthetics, penetrating well into nervous and fatty tissues. They are frequently nephro- hepato- and cardio-toxic.

[↓] a hydrocarbon radical, C₅H₁₁.

[♦] CH₂=CH-CH₂-

1.1.3.1.4. Food

Food can be toxic (mushrooms, rhubarb leaves), and more chemicals are released when food is cooked. Cooking kills micro-organisms that could make preserved food, in particular, toxic (botulism), and it also makes many foods more palatable. Since it helps breakdown cell membranes, it makes foods more digestible and more easily absorbed.

More than a thousand low molecular weight compounds are produced when food is heated, mostly at ppm levels, but the toxicity of these chemicals has not been established.

For example, a cola drink uses caramel color produced by heating sugar and ammonia, and contains 138 µg of 4-Methylimidazole, 4.8 times greater than California's 29 µg-per-day limit, computing to a lifetime risk of cancer of 5 out of 100,000 people.

Other potentially carcinogenic chemicals that have raised concerns in the past include polycyclic aromatic hydrocarbons, N-nitrosamines, aromatic amines and acrylamide.

However, there is no practical way to remove these chemicals from cooked or processed foods, and also there is not enough evidence that they present health risk to humans. In each case, after some time passes, public attention fades away, as (1) we must eat something, (2) we have been cooking for a long time (500,000 years), and (3) the risk is difficult to avoid.

1.1.3.2. Classification by Potency: Toxicity is in the Dose



Toxicants have effects that depend on the amount administered. As Paracelsus said, *the dose makes the poison*. 20 drinks of vodka, taken rapidly on an empty stomach, could kill you.

Toxicants have multiple effects. So, to

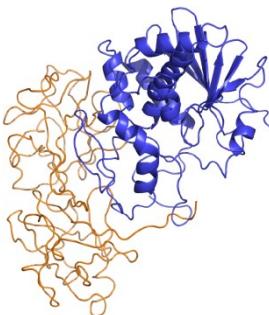
obtain a coherent toxicity classification, it is necessary to agree on the *final outcome by which toxicity will be judged*. The single Lethal Dose that kills 50 % of animals (LD_{50}) has historically been the landmark for the purposes of classification.

Although the determination of LD_{50} implies the death of animals (unethical), it should be considered that almost any chemical manufactured will inevitably intoxicate some human at some time. When this occurs, knowledge of the LD_{50} allows immediate evaluation of the gravity of the intoxication, a considerable advantage in clinical treatment.

Thus, toxicants can be classified according to their *potency*, the weight that will produce a given response per animal body weight, otherwise known as the *specific dose* (T1.9).

LD_{50} generally refers to death of an animal, but it could also refer to death of a cell. For example, a *single molecule* of ricin (shown, from castor seeds, $LD_{50} = 2\mu\text{g/kg}$) reaching the cytosol has been reported to kill a HeLa cell as a result of protein synthesis inhibition. Ricin causes lethal damage to the gut if ingested, and to the lungs if inhaled. A vaccine has been developed against this toxicant (antibodies can neutralize it).

A whole class of bacterial secretions, the bacteriocins, are lethal at a dose of a single molecule per cell. Interestingly, the producers of bacteriocins also synthesize a second protein that pairs up with the antibiotic, keeping it inactive until it reaches its target.



AGENT		LD_{50} , mg/kg*
Ethyl alcohol	SLIGHTLY TOXIC	10,000
Sodium chloride		4,000
Ferrous sulfate	MODERATELY TOXIC	1,500
Morphine sulfate		900
Phenobarbital sodium	VERY TOXIC	150
Picrotoxin	EXTREMELY TOXIC	5
Strychnine sulfate		2
Nicotine		1
<i>d</i> -Tubocurarine	SUPER-TOXIC	0.5
Hemicholinium-3		0.2
Tetrodotoxin		0.10
Dioxin (TCDD)		0.001
Botulinum toxin		0.00001

T1.9 Potency Rating Chart using acute LD_{50} s.

LD_{50} is the dosage (mg/kg body weight) causing death in 50 % of exposed animals.

1.1.4. Limiting the Consequences of Intoxication

Intoxication is dealt with by

- (1) removal of the toxicant,
- (2) elimination of the toxicant from the body,
- (3) treatment with an *antidote, antagonist, metabolic modulator or substrate*, and
- (4) allowing the body's own regenerative powers to take over.

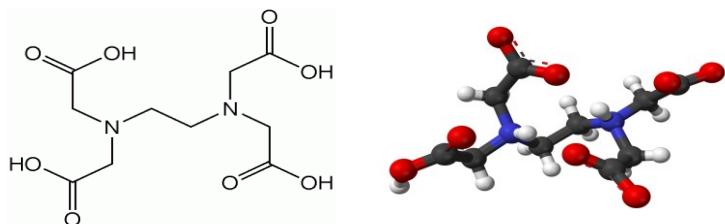
1.1.4.1. Eliminate the Toxicant from the Body

Once absorbed into the body, can toxicants be taken out? If the skin has been exposed, it can be washed. The lung can take in toxicants, but it can also exhale them.

Emesis, the regurgitation of a poison taken via the oral route, can be induced by *syrup of ipecac*, but this must be done within tens of minutes to be effective.

If an ingested poison cannot be vomited, perhaps it can be bound within the *enterum* using a high performance *absorber* such as *carbon black*, which provides a binding tight enough that the toxicant is chaperoned out of the body in the feces. Typically, charcoal given at 10 g of charcoal per gram of toxicant will absorb 50 % of a poison, even 1 hour after ingestion. The list of toxicants that are bound by carbon black include: cyanide, malathion, parathion, diazinon, dichlorvos, DDT, carbamates, mercuric chloride, methanol, N-methyl carbamate, ethylene glycol, kerosene, turpentine, isopropyl alcohol and tolbutamide. It does not bind lithium and potassium, iron or lead.

If the toxicant has escaped from the digestive system into the blood, perhaps injection of a *chelator*, able to specifically bind the toxic agent in the blood and help carry it out of the body, is possible. For example, injections of the chelator ethylene-diamine-tetra-acetic acid (EDTA, shown) have been the method of choice for the treatment of lead intoxication.



In most cases, toxicants bound by *chelators* can be excreted from the body via the natural routes, mostly the liver and kidneys.

For many chemicals, the slow process of clinical recovery is closely related to elimination of the agent from the body, often monitored using blood plasma levels (Chapter 4, Toxicokinetics).

But some injuries cannot be cured by elimination of the toxicant. In burns, for example, the injurious agent dissipates completely following injury, but leaves behind manifestations such as edema, intravascular coagulation, small vessel thrombosis and ischemia, all this in the absence of the agent itself. This underscores that the *agent* itself and its *effects* can have different *half-lives*. More will be said on the elimination of toxicants in Chapters 4 and 8.

There are basically two types of biological monitoring in toxic reactions: following the evolution of the toxicant concentration over time, and assessing the evolution of various aspects of the toxic injury. These two aspects are often linked.

1.1.4.2. Treatments or “Antidotes”

Some toxicants act by inhibiting or over-stimulating the nervous system. In this case, if appropriate chemicals can be ingested or injected, the effect of the toxicant may be compensated or at least blocked long enough for the toxicant to be naturally eliminated. As well, some toxicants target molecular steps in metabolism: inhibition of enzymes, depletion of vital substrates, de-activation of critical molecular species. Occasionally, there are effective answers to such intoxications that can reverse the toxic effects. A number of examples are presented in Table 1.10.

1.1.5. Specificity of Toxicants

As a rule, simpler toxicants are likely to have more widespread effects among species and body systems.

T1.10. TOXICANTS and...		ANTIDOTES
		BY SPECIFIC BINDING TO THE TOXICANT
Botulism	<i>Botulinum</i> antitoxin	
Scorpion, spider, rattlesnake	Specific Antivenins	
		BY GENERAL BINDING TO THE TOXICANT (CHELATION)
Arsenic and mercury	Dimercaprol, D-penicillamine	
Iron	Deferoxamine	
Lead	Calcium ethylenediamine tetra-acetic acid (EDTA)	
		BY BUFFERING OR NEUTRALIZATION
Chlorine gas	Nebulized sodium bicarbonate	
Heparin	Protamine	
		BY COUNTER-ACTING EFFECTS ON CELLS (ANTAGONISTS)
Organophosphates and carbamates	Atropine	
Opiates	Naloxone, nalmefene (opioid antagonist)	
Dioxin	Epigallocatechin gallate (green tea leaves)	
Benzodiazepines	Flumazenil	
		BY MODULATION OF METABOLISM
Acetaminophen	N-Acetylcysteine (increases glutathione)	
Methanol	Ethanol (slows metabolism of methanol)	
Methemoglobin-forming agents	Methylene blue (reactivation of Hemoglobin)	
Methotrexate	Folate (vitamin B9, rebuild folate levels), leucovorin	
Isoniazid, monomethylhydrazine	Pyridoxine (vitamin B6, rebuilds GABA levels)	
Anticholinergics	Physostigmine (cholinesterase inhibitor)	
Warfarin anticoagulants	Vitamin K (restores levels depleted by warfarin)	
Ethylene glycol	Ethanol (slows metabolism of ethylene glycol)	
Ethanol	Thiamine (vitamin B1, Wernicke-Korsakoff syndrome)	
Hypoglycemic agents and insulin	Dextrose (restores blood sugar)	
		BY CREATION OF AN EXCRETION ROUTE
Cyanide	Amyl nitrite, sodium nitrite, thiosulfate	
Hydrogen sulfide	Sodium nitrite, thiosulfate	

1.1.5.1. Wide-Ranging Toxic Effects

- ⊕ For example, for embalming bodies, it is necessary to kill or halt the progress of a wide range of microorganisms. For some time, arsenic trioxide was used for this purpose, until it was replaced by formaldehyde (5-35 %), glutaraldehyde, methanol or ethanol (9-56 %).
- ⊕ Insects are vulnerable to many of the traditional pesticides because most of their bodies or food supplies are being sprayed, while humans are hopefully exposed to relatively

small quantities. The careless human handler can experience life-threatening consequences if given sufficient dose, just as the insect: here again, the toxic effects are relatively non species specific.

Other toxicants depend on particularities of tissues or species, and are species-specific. Protein toxicants are more likely to be specific.

1.1.5.2. Specific Toxic Effects

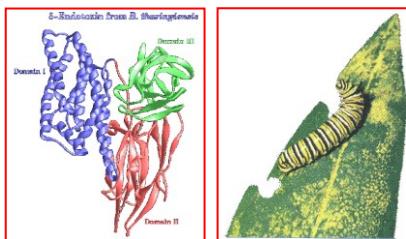
- ⊕ Aspirin can kill cats and acetaminophen snakes, while guinea pigs can consume strychnine, which is very poisonous to humans.
- ⊕ Diclofenac was used to treat swelling and pain in cattle in India. As the drug became popular, the population of vultures decreased by 95 %, as the vultures eating cattle carcasses died of kidney failure²⁴. Diclofenac is being replaced by another non-steroidal anti-inflammatory drug, meloxicam.
- ⊕ *Allelopathic* plants such as the black walnut, sycamore and sassafras trees release substances in the environment through their roots, leaves or by evaporation that limit the germination of competitors.
- ⊕ The toxicant specificity can involve very recognizable mechanisms: the spotted knapweed of the American west secretes catechin, which in tiny amounts induces apoptosis in other plants.

1.1.5.3. Bt-Toxin

Ideal pesticides would affect only a target species (the pest) while leaving the human handlers unharmed. Recently, a controversy erupted concerning the *Bt-toxin* from Monsanto, a molecule specifically lethal to corn borer caterpillars by perforating their gut. Derived from the bacterium *Bacillus*

thuringiensis, it was bio-engineered into a corn seed that makes its own pesticide (F1.11). An unexpected toxicity was discovered: in sufficient quantities, pollen from Bt-plants also kills larvae of monarch butterflies.

Most toxicants are also tissue specific, presenting more risk to certain organs than to others. Lead stored in bone, and organic solvents stored in fat do not cause functional damage, but both can seriously affect the nervous system, if they reach it.



**F1.11. Structure of Bt-toxin.
A monarch caterpillar dines on a
milkweed dusted with corn
pollen.**

1.1.5.4. Side-Effects

The problem of agent specificity is also important in pharmaceuticals, where “side-effects” are common. Some side-effects such as rashes, fever, fatigue and nausea are mild, but some, like internal bleeding, reduction in white blood cell numbers and abnormal heart rhythms, are serious and can result in hospitalization and death.

- ⊕ Class “A” side effects are an exaggerated therapeutic response.
- ⊕ Type “B” side effects are unpredictable from known pharmacology.
- ⊕ Type “C” side effects affect something else than the intended target and are often caused by a metabolite of the drug.

About 60 % of drugs known to trigger adverse reactions do so because the patient metabolizes them too slowly, lengthening the effective time of exposure.

In the case of drugs that *need* to be metabolized to be active, the patient may get no benefit if metabolism is too slow. Conversely, if metabolism is too fast, the patient overdoses.

1.1.6. Definition of Dose

Toxicologists assume that detection of adverse biological effects in animal models increases the chance that the same effects occur in humans. But do the effects start at the same threshold dose?

Although the most common way of specifying dose is weight of substance per weight of living subject (for example, mg/kg), this metric is not necessarily the most useful.

In 1883, Max Rubner pointed out that between animals of different size, mass increases according to Length³ (or Mass¹), while surface increases according to Length² (or Mass^{2/3}).

Under the assumption of complete and uniform penetration of toxicants in a passive body, if one wished to obtain a constant toxicant concentration at the action sites, dose should be proportional to Mass to the first power (Mass¹).

Imagine now the *dynamic* absorption of the toxicant through the body. The concentration at the action site will be primarily determined by transfer across body membranes, for example the inside of the lungs, digestive system, or the inside surface of the vascular system. This surface transfer scales according to surface or Mass^{2/3}.

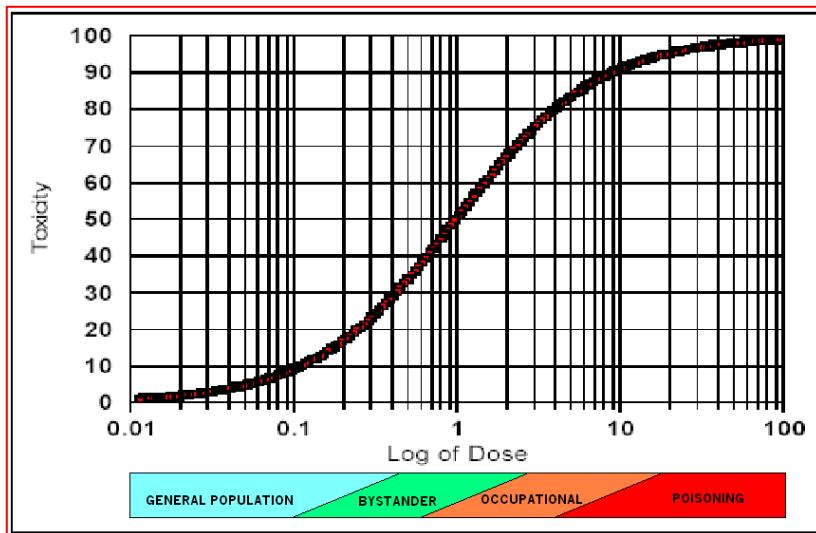
In 1932, Max Kleiber looked at a broad range of data from many animal models and found experimentally that metabolic rate was related to Mass^{3/4}. The value of 3/4 falls between 1 and 2/3, and this range is probably appropriate for the bulk of toxic reactions.

Therefore, both geometry and metabolism seem to point to the importance of maximum local concentrations as opposed to long-term bulk averages in determining toxicity.

Refinements are possible in the definition of dose. Since the molecular weight of the toxicant is not a factor in the expression of toxicity, perhaps an even better metric would be the number of moles of toxicant (= a number of molecules) per mass of animal to the power 0.75.

Other scaling relations have been observed. An animal's lifespan scales as $\text{Mass}^{1/4}$. Heart rates scale as $\text{Mass}^{-1/4}$ (F1.14).

However, the widespread practice is to use a simple metric of mg/kg for dosage. It is simple, and toxicologists feel they have other more substantial inaccuracies to deal with in the conduct of their tests.



F1.12. Ranges of chronic toxic exposures in human populations.
General Population, Bystander, Occupational, and Poisonous exposures cover broad ranges, with overlaps.

1.1.7. Levels of Exposure

1.1.7.1. High Exposures

Victims of accidental or suicidal poisonings, occupationally exposed workers, bystanders (workers nearby, those living close to an industrial facility emitting toxic agents) and the general public typically have different exposures to a particular agent, as shown in F1.14.

It is easiest to document toxic effects at the highest doses, where symptoms of toxicity are easily and rapidly detected. If no discernible adverse health effects can be detected acutely (within a day) at high exposures, it is conveniently assumed that no life-threatening effects will be observed at lower levels over short periods.

1.1.7.2. Low Exposures

Significant low-exposure impacts of pesticides, for example, are being discovered regularly. At a concentration of 15 ppb in the Sierra Nevada, the pesticide endosulfan, widely used and drifting from California, killed a number of frog species. In some frog species, the LD₅₀ is only 0.3 ppb²⁵.

1.1.7.3. Ranging across Exposure Levels

Documenting acute effects, such as the **Lethal Dose to 50 % of animals** (LD₅₀) is the first step in assessing safety. This value is known for a wide range of chemicals, in part because experiments are not too expensive.

Although LD₅₀ levels are very important in guiding dosage for progressively longer tests to be performed subsequently at lower levels, toxicologists are most concerned with identifying toxic reactions that occur at the smallest exposures (**Lowest Observed Adverse Effect Level** or LOAEL), because of the importance these levels have on ultimately determining safe limits over long periods of time in human populations.

1.2. The Principles of Toxicology

1.2.1. Determinants of Toxic Action

The basic principles of Toxicology attempt to give a theoretical framework for the understanding of toxic reactions, essentially defining the *factors that will influence the development of toxicity*.

1 - TOXICANT REACHING THE VULNERABLE SITE

Molecular size, hydrophobicity-hydrophylicity and ionization of the toxicant mostly determine repartition within body compartments. The toxicant exerts detectable effects if it reaches these body compartments.

2 - TIME-COURSE OF TOXICANT DELIVERY

Whether the same amount of a toxicant is administered as a single bolus, or spread over decades, will influence strongly the type and intensity of the toxic reaction. How the body handles the toxicant by mechanisms of elimination is the subject of *Toxicokinetics*¹.

3 - ACTIVATION OF DETOXIFICATION MECHANISMS

Living systems are adaptive, and deal with weak toxic exposures by maintaining *homeostasis*. Therefore, weak toxic exposures can be accompanied by a tissue reaction to intoxication for a certain period of time. These changes may be related to stress protein responses, DNA and cell repair mechanisms, and apoptosis (culling of marginal cells).

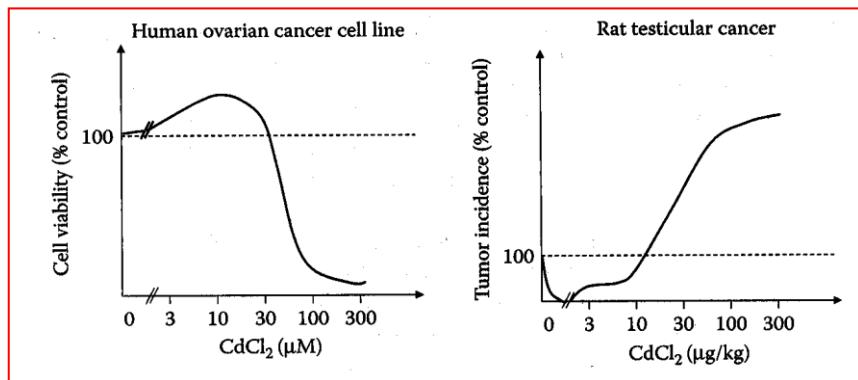
Hormesis

A 19th century view by Arndt is that weak stimuli slightly *accelerate* vital activity, middle-strong stimuli *raise* it, strong ones *suppress* it and very strong ones *halt* it. Schultz published at that time many examples of yeast growth *stimulation* by subtoxic concentrations of various poisons (iodine, bromine, arsenious acid). The *Arndt-Schultz law* says that every stimulus on a living cell produces an activity inversely proportional (within limits) to the intensity of the stimulus⁵. This modulation with dose of the vital reaction is the *inverse* of the conventional dose response.

Certainly, low doses of toxicants awaken responses in the immune system. A single dose of an antitumoral immunosuppressive substance (cisplatin) is able to induce *increased* lymphokine-activated killer activity⁶, and low doses of cytostatic agents *stimulate* human granulocytes and lymphocytes growth⁷. Southam and Erlich⁸, who reported the stimulatory effect of an antifungal when used at low doses, proposed the term "**hormesis**", defined as the *stimulatory effect of sub-inhibitory concentrations of toxic substances*. Stebbing⁹ developed the concept with other scientists.

Hormesis, a non-specific phenomenon increasing the resistance and growth of all living organisms, may be frequent in pharmacology and toxicology. Hormesis relating to a number of endpoints, such as growth, learning, reproduction, birth defects and cancer is seen in organisms from bacteria to humans, caused by a variety of chemicals, including lead, cadmium, mercury and dioxin. Many *stimulants* are toxic at high doses.

¹ Toxicokinetics is the mathematical description of the elimination of toxicants.



F1.13. Protective response at low doses (hormesis) can be illustrated either as an increase in cell viability (left) or a decrease in tumor incidence (right). Calabrese and Baldwin, 2001.

The existence of hormesis raises questions²⁹ about

- (1) the use of high-doses in tests to predict what happens at low-doses, specifically about using the maximum-tolerated-dose in testing for carcinogenesis,
- (2) the use of certain chemotherapeutic drugs. The decay of a drug's concentration over time may produce a long tail at a concentration that stimulates the proliferation of the surviving cells. The tumor eradication potential of the drug may be partly attenuated by proliferation stimulation.

Action of Caffeine vs Dose

Caffeine has never been strictly approved as a pesticide, but it can be used as a 2 % solution to kill frogs. Caffeine consumption in pregnant women should be limited, to protect developing brains.

Stimulation (+)

Two cups of coffee, blood caffeine level of 1-10 µg/ml.

Seizures (-)

Have been reported at 50 µg/ml.

Death (- -)

Reported as low as 80 µg/ml.

Adaptation to Toxicants

This "action-reaction" mode of hormesis, coupled with the synthesis of specific defense molecules in reaction to mild intoxication, may explain why sub-lethal doses of toxicants trigger *adaptive responses* which maximize the innate poisoning resistance of living organisms³⁰. When a strong challenge is subsequently applied, the toxic reaction is attenuated. Mithridate[®] and Rasputin are celebrated users of this technique.

This response, because it includes time for adaptation, is likely different from hormesis, but is similarly effective against toxic effects. Some believe that low doses of synthetic and natural chemicals are actually beneficial, and reduce cancer rates by potentiating defense responses.

In other cases, low doses may lead to a subtle tolerance that is unwanted. It is believed that the repeated mild exposures to drugs that anesthesiologists are subjected to while performing their functions (they are frequently in the breathing zone of patients, where intravenously administered drugs are exhaled by the lungs) may make them more vulnerable to developing drug abuse problems²³.

Tachyphylaxis designates a rapid decrease in the response to a drug after repeated doses over a short period of time. General explanations for the phenomenon: depletion of intermediates creating the drug's effect, or depletion of the drug's receptors in response to their saturation. The best known examples are from street drugs such as amphetamines, ecstasy and ephedrine.

[®] King of Pontus, enemy of Rome, nibbled a mix of 54 ingredients to protect himself against poisoning. When he actually attempted to poison himself, as the Romans were closing in, he failed, and had to die by the sword.

A tempting view is that all intoxications are basically *non-threshold*, but that thresholds are observed experimentally because of lumped reactions that include detoxification mechanisms and hormesis together that add their contributions to the *non-threshold* curve.

4 - TOXICANT INTERACTIONS

Multiple toxicants in combination may have effects unpredictable from their individual actions. Primarily because of interactions documented in the liver (cytochrome enzymes), it is known that two toxicants can have combined toxic effects that add

- ✚ synergistically (more than the sum),
- ✚ linearly (the sum) or
- ✚ antagonistically (less than the sum).

For example, administration of ethanol is used to attenuate ethylene glycol (anti-freeze) toxicity. Toxicants that act by generating free radicals or causing cancer, such as dioxins, furans and PCBs are generally assumed to have additive risks, although it is relatively rare that solid data is available to confirm the assumption²¹.

5 - TIME-COURSE OF TOXIC REACTIONS

Toxicodynamics investigates the changes over time in the physiology and structure of an organism as a result of toxicant exposure.

Positive toxicodynamic change: the rise in the level of CYP enzymes in liver cells, to allow faster clearance of a toxicant.

Negative toxicodynamic change: elimination of healthy cells from tissue as a result of necrosis or apoptosis.

Positive or negative toxicodynamic change: up- or down-regulation in the nervous system.

Toxic reactions will develop and repair at different rates, depending on whether the lesion is metabolic, structural, genetic or heritable.

Short time-frame: metabolic poisons such as cyanide.

Long time-frame: liver inclusions of fat and fibroblasts from exposure to alcohol.

Very long time-frame: genetic changes in the soma (cancer of individual) and in the reproductive cells (changes in the offspring).

Toxicities can cumulate over time, as a consequence of the time-course of toxicant delivery (Principle 2), or attenuate over time, as a consequence the activation of detoxification mechanisms (Principle 3).

Cumulation: heritable genetic damages from ionizing radiation.

Attenuation: increased number of detoxification pumps in the membrane of cells to enhance elimination of low levels of toxicants.

1.2.2. Modeling principles

For reasons of ethics and cost, toxicology is heavily dependant on models in securing toxicity data. While maintaining the representativity of the test, the toxicologist seeks:

- ✚ to use a lower species or test system,
- ✚ to obtain results faster, and at lower cost.

1 – FIND A RELEVANT MODEL

The intent of toxicology studies is often to determine Safe Human Doses such that *no adverse effect occurs in human subjects*.

Because of limits in our knowledge, it is not possible to reliably create toxicological models that exactly determine human toxic thresholds, and the corresponding safe toxicant exposures.

The next best strategy is to use toxicological representations that acknowledge less precision, but that include *Safety Factors* to compensate for our ignorance. The following factors contribute to the imprecision of toxicological models.

A- DESPECIATION

Species have enough similarities between them to make animal or cellular models from many species acceptable, but it is difficult to certify that the system chosen is appropriate for the determination of a particular toxic threshold.

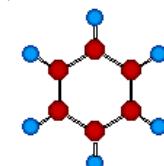
Anatomical, micro-anatomical and molecular-genetic similarities must be considered in the choice of appropriate models for toxicity testing. Selected test species should ideally display the same natural diseases, syndromes or toxicities as humans. Models of tissue cultures, cell cultures, or even biochemical mixtures can meaningfully represent complete living systems only if they are chosen wisely based on previous physiological knowledge. Most of the differences are quantitative, but in a game of toxic thresholds, these quantitative differences can be important.

Specific variables among a broad range of mammals have been studied. Basal metabolic rate (BMR) dependence on body mass (M) in mammals is generally expressed as $BMR = aM^b$, but the value of b is uncertain. Surface area-to-volume ratio argues for geometric scaling ($b = 0.67$), while others claim a quarter-power scaling ($b = 0.75$) supported by theoretical analyses of

nutrient supply networks. These simple views do not account for the shared evolutionary history of some species, who may share metabolic characteristics. b differs among lineages, ranging mostly between 0.67 and 0.75, and there may be no universal relationship between mammalian BMR and M .²⁸

1.2.2.1. Good Animal Models

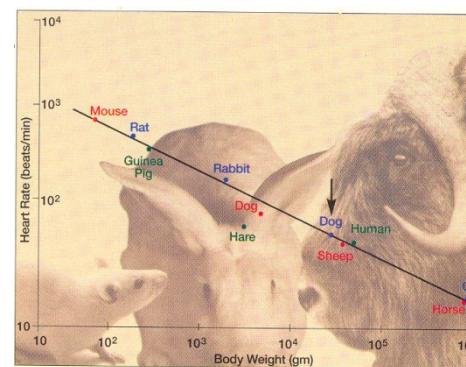
Animal models may need only **small adjustments** to compensate for inter-species variations. For example,



(1) the hematopoietic toxicity observed following exposure to benzene. The mechanism is the same in most mammalian species, but some models are more sensitive than others,

(2) some of the neuropathic conditions in humans attributed to pesticides and solvents are faithfully reproduced in certain animal species. The adult chicken is a good model for

neuropathy from some organophosphorus ester insecticides and hexacarbon.



F1.14. Among animals, heart rate tracks weight with a 0.25 power law. A dog about 1/16th of the weight of a horse has a pulse twice as fast.

1.2.2.2. Poor Animal Models

Some animals models may be **inappropriate** because they lack the biochemical machinery that makes another species vulnerable (such as the lack of thyroid binding protein in rodents versus humans, see Chapter 10).

1.2.2.3. Inter-Species Barriers

About 60 % of infectious diseases are shared between humans and animals (zoonosis), a testimony to similarities between them: bird flu, **SARS**, **HIV**, **Ebola**, bubonic plague, **yellow fever**, **monkey pox**, bovine tuberculosis, Lyme disease, **West Nile** fever, **Marburg**, strains of **influenza**, **rabies**, **Hantavirus** pulmonary syndrome and **Nipah**. Most (in bold) are due to viruses. Smallpox does not have an animal reservoir, and this is why it could be eradicated.

Inter-Species barriers prevented migration of scrapie-like diseases to humans from sheep, but bovine spongiform encephalopathy (BSE) did migrate somewhat to mink and humans... These barriers manifest subtle differences between models that look superficially equivalent.

Species variations problems may be overcome by testing in numerous animal species, or by an *appropriately chosen* single model.

However, the consensus appears to point to an unavoidable imprecision in substituting a model for the real thing, this imprecision being set as a safety factor of 10 in human toxic threshold determinations.

B- CHOOSE RELEVANT VARIABLES

There are innumerable end-points that can potentially be chosen to quantify toxicity, and finally to determine toxic thresholds (smallest adverse effect level). They range from death of a subject (this would be used with extrapolation to lower doses) to, say, increases in the concentration of ornithine decarboxylase enzyme in cultured cells.

How can we be sure that the toxicity variables we have retained are appropriate to quantify the human toxic threshold, and that, for that matter, our choice of variables was wise in the context of all possible human adverse effects, especially the

chronic ones? Does the test display effects on the most sensitive target within the organism? Most tests can find a connection between an exposure and an effect, but is this effect appropriate to determine the acceptable human exposure levels? In the absence of complete knowledge, many toxicologists believe that a safety factor of 10 is appropriate to cover this uncertainty.

C- CHOOSE APPROPRIATE TEST ENVIRONMENT

Once we have selected the proper species for representation of the human toxic threshold, and have decided on the variables to represent such an effect, how do we know that the *circumstances under which the tests will be conducted* will result in a safe human dose that will guarantee safety?

Would you have predicted that in some animal models (but not in others) the baseline incidence of liver tumors can increase solely as a result of animals being fed a choline-methionine deficient diet?

Do we know that, within a given species, we have chosen the most appropriate strain, subspecies or genetic variant for this toxic threshold determination? Could our determination be influenced by age, sex, route of entry, the test's environmental parameters or the culture medium in the case of *in vitro* tests? In the absence of complete knowledge, many toxicologists believe that a safety factor of 10 is appropriate to cover this uncertainty.

It has not escaped your attention that all of these limitations of models (safety factors of 10) can weight heavily in the direction of reducing safe human doses to very small values, potentially making them difficult to implement in practice.

2 - LOW-DOSE EXTRAPOLATION

Toxicology is in a hurry to get results. Low exposures in humans are often simulated by high doses in an animal model, under the assumption that high doses in animals will produce the same toxic reaction as observed at low doses in humans, but with more statistical strength (*binding isotherm* view, see Chapter 2).

To reduce the number of animals necessary for a test, while still obtaining robust data, extrapolation from high to low doses is frequently used.

The reliability of toxicity testing therefore often depends on a monotonous relation between the log-of-dose of the agent and the toxic outcome. Although this assumption is almost universally used, there is substantial evidence that there are significant departures from it, as elevating exposure changes the diseases expressed in a model from the chronic class to the acute class.

1.2.2.4. Difficulties of Low-Dose Extrapolation

- ✚ Patterns of toxicant distribution and metabolism differ between low and high doses.

Using high exposures in experiments tends to target the organ with the weakest elimination (short-term bioaccumulation). This organ then becomes the determinant of the TLV, although it may be the wrong target for chronic exposure.

- ✚ Defense mechanisms, active at low doses, may be disabled at higher ones.

A low dose may be compensated by normal physiological mechanisms, only a small fraction of the toxicity being expressed in the model, as much of the toxicant is effectively neutralized in a healthy subject.

1.2.2.5. Improving the Models

If exposure must remain low in the model to conserve mechanisms, can the model itself become more sensitive to reduce testing costs?

Toxicity amplification, other than by increasing dose, may be possible.

- ✚ For example, by reducing the reserve of detoxification molecules and antioxidants. But it is not clear that the effects are equivalent to increasing dose with none of the disadvantages.
- ✚ In animals, we can enhance the sensitivity to chronic diseases by genetic knockout techniques (Chapter 10). This approach may be meaningful for genetic mechanisms, but not necessarily for toxicity generally.

Example of Low-Dose Non-Linearity

Ionizing radiation risk is in great part based on health outcomes from the Hiroshima and Nagasaki explosions, and assume that cancer risk is proportional to dose, even at low levels.

In careful experiments, researchers were able to irradiate the nuclei of cells in Petri dishes with one alpha particle each, creating mutations of a specific gene.

Irradiating 20 % of the nuclei produced 80 mutations per 100,000 cells.

Irradiating 5 % of the nuclei produced 57 mutations per 100,000 cells.

Bystander effects due to cell-to-cell communication are thought to account at least in part for the observed non-linearity¹⁴.

3 – LONG-TERM EXTRAPOLATION

This is the assumption that brief tests (days to a year) can represent decades of human life.

Many testing procedures in various areas of technology attempt time-saving measures. In the testing of materials, higher temperature or temperature cycling are often used to increase the aging rate. In the testing of electrical cable insulation, the frequency of the applied voltage can be increased.

In inorganic chemical reactions, concentration (law of Mass Action) or temperature (Arrhenius equation) can be increased to augment reaction rates.

These tactics cannot be used in toxicological models.

Biological molecules have limited tolerance to heat and pH. Molecular components of living systems, particularly protein, react and denature differentially under altered conditions (temperature, pH, concentration).

1.2.2.6. Accelerating the passage of Time

There are however acceleration methods that are applicable to biological models:

- + increasing the cell cycling (division) rate,

There is a generally higher susceptibility to toxicants by organisms hosting rapidly dividing cells (fetus, bone marrow, immune system).

- + cancer initiation-promotion,

In cancer research, promoters can be introduced to illuminate and amplify the action of initiators, and vice-versa.

- + genetic susceptibility.

Animal models can be created with genetic susceptibility to specific pathologies (for example, with one copy of p53 disabled, see Chapter 10).

However, most (brief) toxicological tests performed are *assumed* with relatively little proof to be representative of risks developing over much longer periods. Within the duration of a

2-year chronic animal test, animals go through maturation, gestation, adulthood and aging, generally assumed to correspond to segments of human life.

1.2.2.7. Choices in Toxicology

In the thinking of toxicologists, low-dose extrapolation is thought to amplify the risk because more mechanisms of toxicity are thought to be active at high doses than at low doses.

To the contrary, long-term extrapolation is thought to attenuate the risk because it does not take into account bio-accumulation of agents, or slowly emerging damage.

Compensating one factor with the other, toxicologists often simulate chronic low level exposures in humans using short high level doses in an animal model.

Taken together, low-dose extrapolation and long-term extrapolation pose formidable credibility problems for toxicology. Would an athlete look the same after training forcefully for 6 months as if he took it easy for 3 years ? Therefore, a basic problem of Toxicology can be stated as:

Time does not respect what is done without it.

1.3. Dose and Time in Toxicity

High doses of toxicants generally have rapid effects, whereas effects of lower doses may become detectable only much later. This makes sense, since many physiological processes accelerate when concentrations increase.

Since acute toxicity determinations are relatively simple, fast and inexpensive, they form relatively little of the ongoing activity in Toxicology.

A class of natural compounds with considerable acute toxicity are venoms. They are rare in mammals, but very common in

lower life forms (snakes, spiders, anemones). The proteins and peptides of venoms act rapidly.

Venoms target the blood and blood vessels, inhibiting clotting and dilating vessels, leading to a loss of blood pressure. They also target nerves and muscles by acting on calcium, potassium and sodium-channel blockers, as well as sodium-channel activators and prolongers and antagonists of nicotinic and muscarinic receptors. Venoms are probably derived from the immune system's β -defensin molecules, and are usually represented in numerous types within the same animal to maximize effectiveness.

By contrast, considerable effort is expended on elucidating more subtle effects that occur slowly at lower exposures. These more common exposures, without being an immediate threat to survival, may produce subtle alterations, with delayed impacts on chronic survival or organ function. Teratogenicity and carcinogenicity are such "subtle" effects. Those may develop after some period of time, following a single high-level exposure, repeated moderate exposures or continuous exposures to low levels of agents for decades (T1.15).

T1.15. Acute or delayed toxicity may occur after a single high-level exposure. Repeated exposure at low levels may manifest in subtle, chronically developing adverse effects.

EXPOSURE	EFFECTS
Single, High	Acute, immediate symptoms, or delayed toxicity after some indeterminate time.
Repeated, Moderate	Covert (subtle) chronic symptoms, or slow onset of symptoms.
Continuous, Low-Level	Emergence of real risks after some time, or risks merge with general background risks.

1.3.1. Haber's Law

The relationship between *level* of exposure and *time* of exposure is quantified by *Haber's Law*:

$$\text{Toxicity} = \text{Potency} \times (\text{Concentration} - \text{Threshold}) \times \text{Time}$$

where *Toxicity* quantifies the toxic effect,

Potency represents the toxic potency of the agent,

Concentration is the concentration of the agent or dose rate,

Threshold is the detoxification term, and

Time is the duration of agent administration.

According to Haber's Law, any toxic effect is the product of the *Potency* of the agent, its *Time of application* and its *Concentration* minus a penalty term for metabolic detoxification, "*Threshold*".

Although Haber's Law is too simple to account for all aspects of toxic reactions (for example, "*Threshold*" is typically a function of agent concentration, rather than a constant, as we will see in Chapter 4), it explains the assumptions most often used by toxicologists, namely:

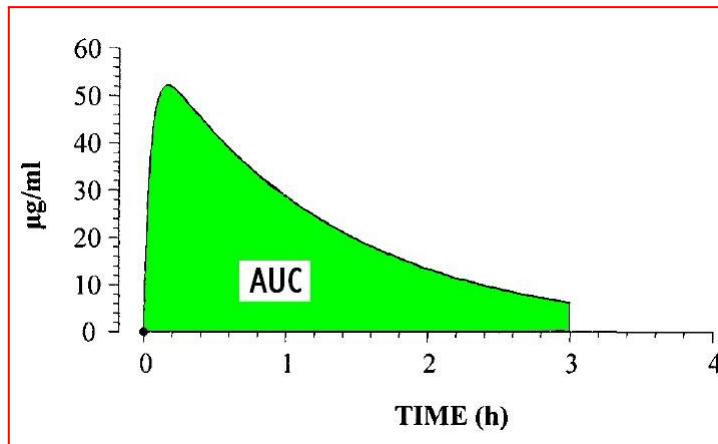
- ⊕ The presence of "*Threshold*" in the equation justifies the existence of a safe exposure. The claim is that *no hazard "exists"* below a Threshold Limit Value (TLV).
- ⊕ Use the "Area-Under-the-Curve" (AUC) to average exposures that vary over time, according to the integration of Haber's Law (below).

$$\text{Time Integrated Toxicity} = \text{Potency} \int [\text{Concentration}(t) - \text{Threshold}] dt$$

Haber's Law is not often referred to in the description of toxicology experiments, in great part because experiments properly measuring exposure over time (as blood

concentrations, for example) are more difficult to perform than the administration of a simple dose. This has lead many to use more descriptive and approximate (as opposed to analytical) methods².

1.3.2. Area under the Curve



F1.16. The “Area Under the Curve” in an exposure vs time plot can be used to quantify toxic effects.

1.3.3. Threshold Limit Values

Nevertheless, the *legal* protection of human health has taken the form of threshold limit values (TLVs) using Time-Weighted-Averages (TWAs), compatible with Haber’s Law. Short-term exposure levels (STELs) have been added to take into account the more acute effects of exposure.

High exposures lead to frank effects on an organism that does not have time to adapt. Low exposures may barely show any effects until long into the future, with the added complexity that the organism may adapt and age as the test proceeds.

THE TIME-WEIGHTED AVERAGE METRIC ONLY APPROXIMATES TOXICITY

While the assumption that the Area under the Curve is the proper exposure metric to gauge toxic effects, it is obviously true only for slow-acting chemicals with straightforward physiological actions.

There are many known exceptions to this rule. For example, suppression of the immune system by diesel exhaust fumes is more effective if smaller doses are repeated at intervals than if one large single bolus is administered²².

In contrast to the case above, most toxicants, if administered in many very small doses as opposed to a single large one, will have negligible effects (because of the Threshold term in Haber’s Law).

1.3.4. Time Course of Toxic Reactions

Toxic reactions unfold over periods ranging from a minute to a lifetime. A toxicant reaches various sensitive sites within the body at various moments, and multiple toxic effects from a toxicant can each have secondary consequences. In some cases, toxicity can be altogether delayed because of the need for bioactivation. Because of basic as well as size differences, toxicities can also happen at different rates in animal models and in humans.

The acute effects may strike an organ or produce a disease totally unrelated to the one that will become apparent as a result of chronic exposure.

Acute effects are generally well known and chronic effects highly uncertain, because acute tests are cheap, while chronic tests are expensive.

1.3.4.1. Toxidromes

From the point of view of a victim, acute toxic reactions follow a certain development over time, depending on the poison concerned. This development is called a *toxidrome*. For a physician, history of the patient is paramount, but the toxidrome (i.e., appearance of symptoms) may provide indications on the compound ingested and on the dose. In occupational and environmental health, the *cholinergic toxidrome* is the most important; other toxidromes (anticholinergic, sympathomimetic, opioid, sedative/hypnotic, hallucinogenic) are most often associated with overdoses of drugs or medications. Interpretation can be complicated if multiple substances are involved.

Imagine a worker exposed to organophosphate pesticides (malathion, diazinon), carbamates (physostigmine, carbaryl), certain mushrooms or the warfare agent sarin. He is a victim of the *cholinergic toxidrome*. Since there are various classes or receptors in the body that could be stimulated in this toxidrome, the symptoms could include any of those described in Table 1.17.

T1.17. Cholinergic Toxidrome

MUSCARINIC	NICOTINIC	CENTRAL
Pupil contraction	Pupil dilatation	Agitation
Slow heartbeat	Fast heartbeat	Confusion
Mucus in lung, obstructed breathing	Bronchodilation	Lethargy
Vomiting, diarrhea	High blood pressure	Coma
Abundant saliva/tears	Perspiration	Seizure
Urinary incontinence	Weakness (resp. arrest)	Death

If a specific toxidrome is identified, the physician should consider toxin-specific treatments, such as an *antidote*.

Antidotes are usually given after the patient is stable, preferably within a few hours of ingestion, and may require multiple doses because of short durations of action.

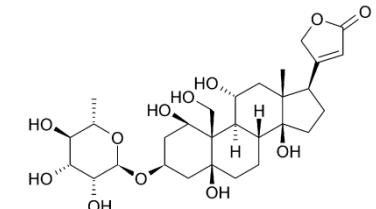
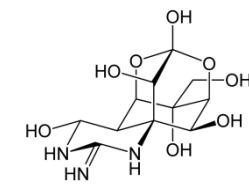
Initial laboratory testing may include bicarbonate level, electrolytes, serum urea nitrogen, and serum creatinine levels to evaluate for renal failure and electrolyte imbalance; blood glucose levels for hypoglycemic ingestion; electrocardiography for cardiotoxicity; prothrombin time for coagulopathy; pulse oximetry for hypoxia; serum acetaminophen level for acetaminophen toxicity; and urine human chorionic gonadotropin levels in female patients of childbearing age.

1.3.4.2. Cell Membrane Toxicity – Minutes

Toxic substances released in extracellular fluids rapidly meet cell membranes. Alterations to the membranes come from altering the state of membrane receptors (neurotransmission, hormones, cytokines), dissolving or altering the lipid matrix, or from binding or cross linking membrane protein. Such toxicities can be established very rapidly, especially for some agents that are quickly absorbed through the lungs.

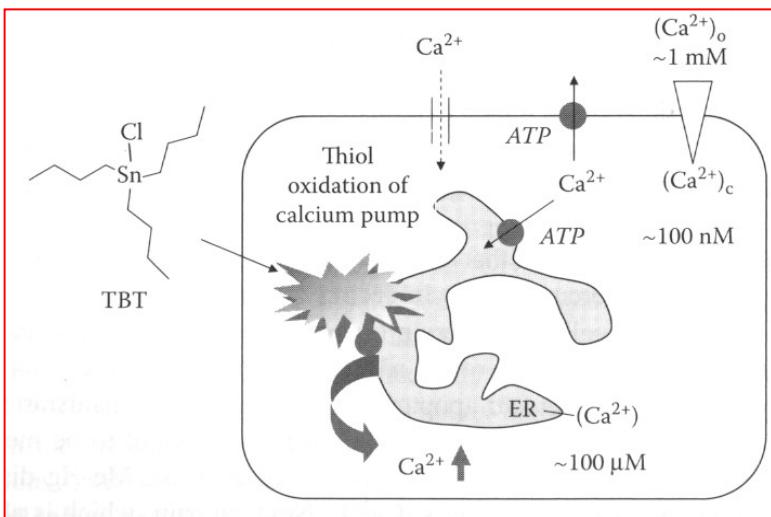
Acetylcholinesterase inhibition, which over-stimulates membrane receptors, is typical of warfare nerve gases such as sarin. Neurotoxicants (DDT, pyrethroid insecticides, tetrodotoxin-shown) either block or alter the function of specialized protein channels for inorganic ions or neurotransmitters.

For example, ouabain (shown) inhibits the cell membrane's Na^+/K^+ pump (ATPase enzyme), eliminating its electrical polarization.



Organic solvents (ethanol, benzene) and anesthetics expand lipid membranes and increase their fluidity and permeability. Oxidation of membrane lipids (overwhelming the protection provided by glutathione) occurs from herbicides such as paraquat and diquat and from antibiotics such as adriamycin and bleomycin.

Calcium in the blood is at a concentration of 0.002 M, but the intracellular resting concentration of free calcium is 10^{-7} M (a ratio of 10,000). Inside the cell, low calcium concentrations are maintained by sequestering calcium in mitochondria, the smooth endoplasmic reticulum and by protein binding (to regulators such as calmodulin). Carbon tetrachloride, acetaminophen and tri-n-butyltin are examples of substances that act by releasing calcium within cells, leading to loss of cell functions and cell death (F1.18).



F1.18. Tri-n-butyltin chloride mobilizes Ca^{2+} from the endoplasmic reticulum, flooding the cytosol. The dark circles are calcium transporters. Apoptosis results. Boelsterli, 2007.

1.3.4.3. Cell Metabolism Toxicity - Hours

Metabolism uses external materials to produce living substance: lipids, protein and nucleic acids. Toxic action occurs by inhibiting materials absorption or bio-synthesis (T1.19).

A few metabolic toxicities occur extremely fast, because they target bottlenecks of cell metabolism. Oxygen deprivation by carbon monoxide suppresses ATP synthesis in the mitochondrion, which can damage the brain within minutes. Cyanide and H_2S also block energy utilization and are extremely fast acting. Milder reductions of mitochondrial metabolism are obtained from the drugs salicylate and tetracycline. Extensive studies have been done on the reaction of mitochondria to a variety of toxicants (T1.20).²

Typically, metabolic toxicity develops a little more slowly than in the extreme examples above, as

- (1) blocking one pathway usually leaves alternatives for cells to compensate with, and
- (2) cells usually have some reserve which allows them to pull through brief episodes of intoxication.

Many components of cells nevertheless need to be replaced, as they wear out through normal metabolism. For example, RNA is synthesized and replaced in cells approximately every day.

1.3.4.4. Cell Proliferation Toxicity - Days

Although most cells *in vivo* are in the resting (non-dividing) phase, some are not. Body tissues with high growth rates are

² About 1 in 5,000 women have mitochondrial mutations that lead to fatal disorders that affect the muscles and the brain. They are passed from mother to child, as mitochondria are maternally inherited. During *in vitro* fertilization, it is possible to move the nucleus of an egg to the cytoplasm of an egg with healthy mitochondria.

bone marrow, small intestine, skin, hair and nails. Any substance toxic to the mitotic apparatus will display cell proliferation toxicity. Methotrexate, an anticancer drug which inhibits DNA replication, is not toxic to cells in the resting phase, but is highly toxic to proliferating cells. This is why people undergoing chemotherapy are subject to hair loss, digestive problems and low blood counts.

Any compound able to alter cell proliferation rate is a potential teratogen, as the shaping of embryos is critically dependant on speed of cell division.

T1.19 . Inhibitors of Cell Metabolism.

RNA only	Actinomycin D
Inhibit protein and RNA synthesis	Streptomycin
	Ethionine
	Dimethylnitrosamine
	Carbon Tetrachloride
	Galactosamine
Protein only	Cycloheximide
	Chloramphenicol
	Puromycin

1.3.4.5. Cell Oncogenicity - Years

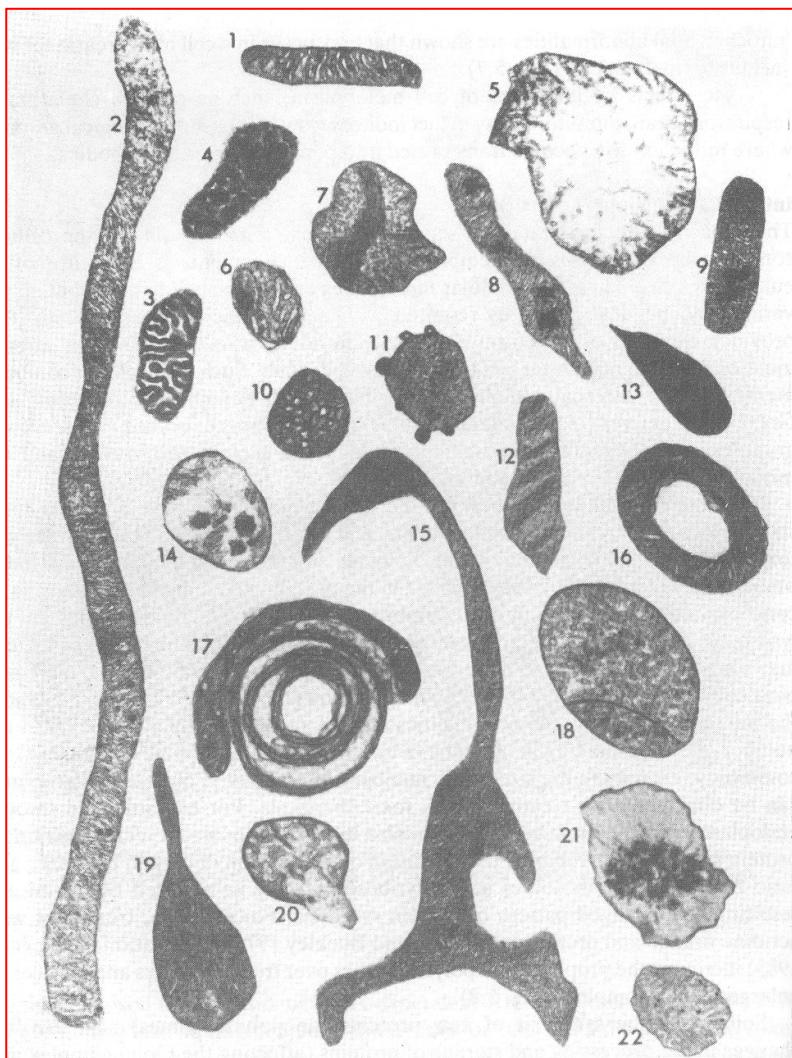
Long-term experiments on toxicity teach us that the longer you study toxicity, the more surprises there are.

Cancer is known as “a multiple step process”, and these steps typically do not all occur within a very short period of time. Even after a tumor cell is created, functionally significant tumors need to grow in order to affect the organism, and be detectable. So, cancer typically develops over years or decades. In rat models, this period of cancer development is compressed within a year.

T1.20. Changes in Mitochondria caused by Toxic substances.

1	Control (no exposure)	
2	Methotrexate	Elongation
3		Condensed
4	Oligomycin	Twisted
5		Swollen
6	20,25-diazacholesterol	Enlarged matrix granule
7	Isonicotinic acid hydrazide	Swollen, collapsed cristae
8	Sodium fluoride	Asteroid inclusions
9	Diethylenetriaminetetraacetic acid	Aggregated matrix granules
10	Puromycin	Vesicular cristae
11	Acetone	Outer membrane blebs
12	Fluoroacetate	Perpendicular stacks of cristae
13	Iodoacetate	Dense matrix
14	Ouabain	Mineral granule accumulation
15	Cyanide	Branching
16	Excess ferric ion	Ring formation
17	Excess calcium ion	Nesting cup formation
18	Dicyclohexylcarbodiimide	Coagulated matricial protein
19	Actinomycin D	Clear matricial area with tail formation
20	Puromycin	Disruption
21	Ouabain	Mineral granule accumulation
22	Cadmium ion	Scalloped outer margin

Mitochondria are former bacteria, and many antibiotics can lead to mitochondrial failure through Reactive Oxygen Species generation. N-acetyl-L-cysteine (NAC) can reduce the damage while maintaining bactericidal action.



Mitochondrial Micrographs, x 25,000. See T1.19.
Birkhäuser Verlag, Basel.

In New Guinea, the central nervous system disease called “kuru” (connected with mad cow and Creutzfeld-Jacob diseases) was related to the practice of ritual cannibalism that included the eating of human brains, which was banned in the late 1950s. According to epidemiological data, this prion-based disease could have an incubation time of 34-41 years²⁷.

1.4. Scope of Toxicological Data

A rounded approach to evaluating toxic risks includes:

- + physical and chemical characterization of the toxicant,
- + *in vitro* studies,
- + *in vivo* studies, and
- + epidemiology.

The types of studies chosen and how they are performed will depend on the **intent of the toxicity study**. Because of cost, acute (short) studies tend to be available in the literature and reasonably well standardized, while chronic (long) studies tend to be few, with more variation in the methods. The evidence available for any toxic agent has accumulated over a period of time, consequently the historical changes in methods and emphasis bring unexpected diversity to an agent's file.

The most inexpensive way to assess the toxicity of a chemical is to do a **literature review**. For example, a review of accidental or suicidal intoxications and of occupational exposures may allow recognition of the acute hazards of the agent.

If literature cannot be found, a modest investment in lab work will yield acute studies of lethality (LD_{50}), dermal sensitization, genotoxicity and cytotoxicity in some models. Such models are well controlled, but there is a problem in extrapolation from the various models to human cases.

Some ***in vitro*** experiments are highly relevant to cancer risks (cell proliferation studies, genetic determinations), while **computer modeling** may be important to compartmentalization (solubility and Toxicokinetics) and gross assessment of toxicity.

The contributions of **computer modeling** and ***in vitro*** studies are primarily in confirming and documenting basic effects and mechanisms. Systematic efforts are being made to expand the scope of these studies to provide more complete information relevant to human risks.

Further experimental work and substantial resources can yield ***in vivo*** genotoxicity, sub-acute, sub-chronic, chronic studies, reproduction studies, etc. Chronic animal experiments are very useful in assessing teratogenic and cancer risks.

All experimental studies have in common the difficulty of establishing appropriate **levels of exposure** for tests. Doses are larger for acute studies than for chronic ones, and there is always a risk in a chronic study of being "off the mark" due to improper dosing.

The usefulness of many **epidemiological studies** is limited by two factors:

- (1) the duration and level of exposure: retrospective studies of worker health typically involve complex exposures, for example as a result of particularities in industrial processes and as occupational health and safety regulations change over the years,
- (2) the simultaneous exposure to a mixture of agents, leading to a difficulty in attributing observed health effects to any individual component.

On the other hand, when epidemiological studies are performed using environments very similar to those that are of interest, their conclusions can be very revealing of real human risks.

1.5. Toxicity Databases

1.5.1. Availability of Toxicity Information

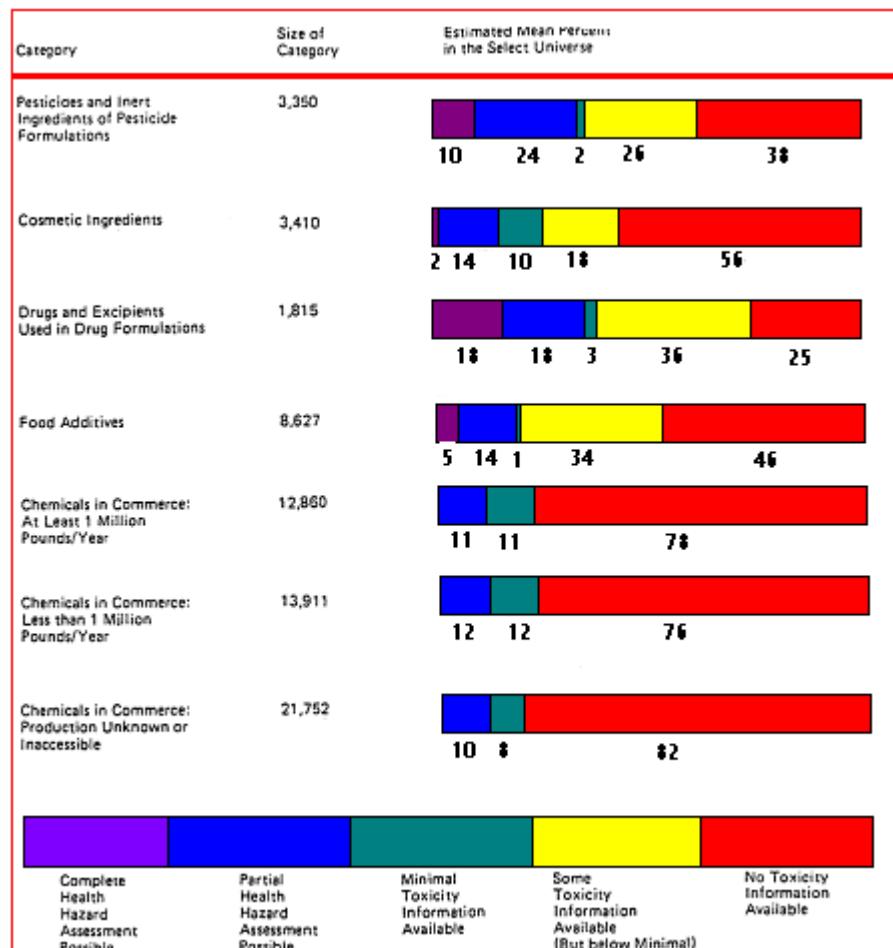
Toxicology will not become decrepit for lack of new chemicals, added at a rate of 36,000 new chemicals to the Chemical Abstracts Service database every day...

Some 65,000 basic chemicals are used in industry to create 5 million compound products. Most of them have been in use for many decades. Few have been re-assessed using the present, more stringent requirements of regulatory bodies¹⁵. These *grandfather* chemicals cause most of the problems.

A study by the US National Academy of Sciences reveals that between 76 and 82 % of chemicals in commerce have too little information to conduct a health-hazard assessment: virtually no toxicity information, as shown in F1.21.

"Adequate" toxicology exists for only 2-3 % of the chemicals. In 1976, the US *Toxic Substances Control Act* established that 60,000 chemicals would be "grandfathered", and that the Environmental Protection Agency would be responsible to perform scattered tests on 100 chemicals, and to restrict the use of 7 % of the chemicals introduced until 1979. This situation has arisen due to:

- ⊕ the absence of demands for testing,
- ⊕ the lack of adequate testing protocols,
- ⊕ the large numbers of chemicals introduced since the 1950s.



F1.21. Availability of health hazard assessments in seven categories¹⁵.

Only a quarter of the 82,000 chemicals in use in the US have been tested for toxicity. Each year, the US EPA reviews 1700 new compounds to be introduced. They are tested only if

evidence of potential harm exists. 90 % of the new compounds are approved without restrictions.

Most of the chemicals in use will have some information on acute toxicity, but virtually none on reproductive toxicity, teratogenicity or carcinogenicity. In the absence of a specific "need to know", the costs and the time required discourage retro-testing.

"Restriction of Hazardous Substances (RoHS)" Compliance

Any RoHS compliant electronic and electronic component is tested for Lead, Cadmium, Mercury, Hexavalent chromium, Polybrominated biphenyls (PBB), and Polybrominated diphenyl ethers (PBDE).

For Lead, Polybrominated biphenyls (PBB), and Polybrominated diphenyl ethers (PBDE), there must be no more than 0.1% of the material, when calculated by weight at raw homogeneous materials.

For Cadmium and Hexavalent chromium, there must be less than 0.01% of the substance by weight at raw homogeneous materials.

Any RoHS compliant component must have 100 ppm or less of mercury, and the mercury must not have been intentionally added to the component.

One may derive the impression, from the above, that there is never enough toxicity information. In some ways, this is true. For toxicological science, it would be desirable to have complete results on all substances. However, after the understanding that the gathering of all potentially useful information is impossibly expensive, the focus shifts to gathering information of good quality and to obtaining the *relevant* information (as opposed to the superfluous).

The strategy is to perform only the tests that need to be done to recognize health risks. A major task of experimental toxicologists is therefore to determine what data is critical to safety, and how this data should be interpreted.

A vital aspect of resource use in toxicology is the use of broad assumptions to qualify categories of chemicals, essentially removing them from expensive experimentation lists because some information is available on similar or connected substances. Although such inferences are not always correct and can lead to spectacular errors, Toxicology often plays the differential science card, avoiding costly experimentation based on acceptance that substances closely related in their physical and chemical properties may share similar toxicity.

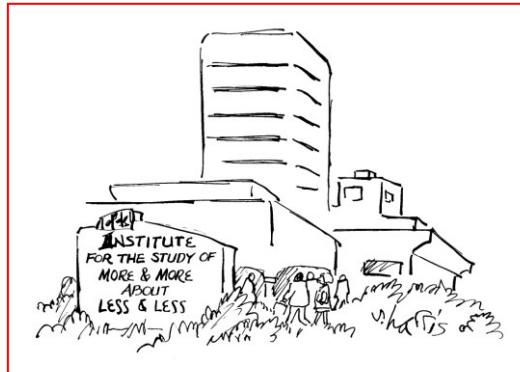
1.5.2. Reliability of Toxicity Information

Agreement among experts is not always a guarantee of safety. Science often proceeds as a self-centered peer-review process (F1.22), which makes it vulnerable. Toxicology, for practical reasons, is more sensitive to toxicities that have obvious manifestations, and that develop over the short term.

Many sciences suffer from this vulnerability: microbiology is biased towards the study of organisms that proliferate well in current culture media, and chemistry favors molecules that are easy to isolate, and are conveniently stable.

F1.22. Sometimes, science can lose perspective.
American Scientist, Nov-Dec 2003.

When new deleterious effects are detected, we often find that they spring from a new



mechanism, involve a new variable, or occur over a longer time frame than previously investigated.

An important line of defense is vigilance on the part of occupational physicians, hygienists, toxicologists and workers themselves, which has contributed significantly to identifying overt as well as covert hazards. The reinvestigation of particular chemicals is frequently driven by a chance observation, a bizarre effect on an exposed individual, or an epidemic intoxication.

In the introductory chapter to a New York Academy of Sciences volume "celebrating" the passage of 200 years since Percival Pott's paper linking soot with scrotal cancer in chimney sweeps, Wagoner made a critical observation¹⁶.

Despite the fact that we know about occupational carcinogenicity associated with coal and oil combustion products, radioactivity, inorganic arsenic, aromatic amines, asbestos, etc., we still have workers exposed to these agents, and they are dying at rates far higher than the normal population. What has been accomplished in these 200 years, if excess exposures still occur?

There is little concern about many of these chemicals, because they have been in use for a long time and are widely distributed. Often, the agent is not connected to the disease, or the disease is difficult to diagnose.

Consider the uranium miners from East Germany and their lung cancers resulting from poor to negligible occupational health practices during decades¹⁷. Lung cancer and disease killed at least 20,000 miners out of a workforce of 450,000 as a consequence of exposure to radiation and dusts.

Consider the neurotoxic disorders, one among a list of 10 leading work-related diseases identified by NIOSH¹⁸. Neurotoxicity can be severe and debilitating, with a large numbers of workers at risk. A vast array of commonly used

chemicals are associated with diverse neurological signs and symptoms, resulting in peripheral and central nervous system damage¹⁸.

Consider the toxicity of lead (Pb) with effects on the nervous system (neuropathy and behavioral-learning deficits), the liver, the kidney and the hematopoietic system.

1.5.3. Regulations on Toxicity Information

Public and worker concerns, encouraged by the media, are demanding more information about chemicals manufactured or imported, distributed across the country by rail, truck or ship, used in various industries, in the home and garden, and disposed of when no longer needed.

National and international regulatory agencies are requiring more extensive data for each chemical entering the marketplace (T1.23). Under current Canadian, US and OECD/EC regulatory requirements, new chemicals must be accompanied by a basic package of certain toxicity tests (varying with the volume being imported or manufactured per year).

The list below is the information requirement for countries of the European Community.

Similar data is required for new chemicals under the Canadian Environmental Protection Act of 1999

(<http://laws.justice.gc.ca/en/C-15.31/text.html>).

Also included at the end of this table is information concerning disposal of the agent, designed both for industry and the individual user, in keeping with the "cradle-to-grave" responsibility of industries for their products.

T1.23. INFORMATION REQUIREMENTS IN SOME EEC COUNTRIES FOR NOTIFICATION AND HAZARD ASSESSMENT OF NEW CHEMICALS

IDENTITY OF THE SUBSTANCE

- Name
- Names in the International Union of Pure and Applied Chemistry nomenclature
- Other names (usual name, trade name, abbreviation)
- Chemical Abstracts Service number (if available)
- Empirical and structural formula
- Composition of the substance
- Degree of purity (%)
- Nature of impurities, including isomers and by-products
- Percentage of (significant) main impurities
- If the substance contains a stabilizing agent or an inhibitor or other additives, specify: nature, order of magnitude:... ppm;....%
- Spectral data (Ultra Violet, Infra Red, Nuclear Magnetic Resonance)
- Methods of detection and determination
- A full description of the methods used or the appropriate bibliographical references

INFORMATION ON THE SUBSTANCE

- Proposed uses
- Types of use
 - ◆ Describe: the function of the substance, the desired effects
- Fields of application with approximate breakdown
 - ◆ closed system, industries, farmers and skilled trades,
 - ◆ use by the public at large, open system, industries
 - ◆ farmers and skilled trades, use by the public at large
- Estimated production and imports for each of the anticipated uses or fields of application
- Overall production and imports in order of tons per year [1, 10, 50, 100, 500, 1,000 and 5,000]
 - ◆ first 12 months
 - ◆ thereafter
- Production and imports, broken down in accordance with fields of application, expressed as a percentage,
 - ◆ first 12 months
 - ◆ thereafter
- Recommended methods and precautions concerning:
 - ◆ handling
 - ◆ storage
 - ◆ transport
 - ◆ fire (nature of combustion gases or pyrolysis. where proposed uses justify)
 - ◆ other dangers, particularly chemical reaction with water.
- Emergency measures in the case of accidental spillage
- Emergency measures in the case of injury to persons (e.g. poisoning)

PHYSICO-CHEMICAL PROPERTIES OF THE SUBSTANCE

- Melting point
- Boiling point (... °C at .. Pa)
- Relative density (D_4^{20})
- Vapour pressure (...Pa at ... °C)
- Surface tension (N/m at... °C)
- Water solubility (mg/litre at... °C)
- Fat solubility
- Solvent-oil [to be specified] (mg/100g solvent at... °C)
- Partition coefficient (n-octanol/water)
- Flash point (... °C in open cup and closed cup)
- Flammability
- Explosive properties
- Auto-flammability (... °C)
- Oxidizing properties

TOXICOLOGICAL STUDIES

- Acute toxicity
 - ◆ Substances other than gases shall be administered via two routes at least one of which should be the oral route. The other route will depend on the intended use and on the physical properties of the substance. Gases and volatile liquids should be administered by inhalation (a minimum period of administration of four hours) in all cases, observation of the animals should be carried out for at least 14 days. Unless there are contraindications, the rat is the preferred species for oral and inhalation experiments. The experiments shall be carried out on both male and female subjects.
- Administered orally (LD50 in mg/kg)
 - ◆ Effects observed, including in the organs
- Administered by inhalation (LC50 in ppm)
 - ◆ Duration of exposure in hours
 - ◆ Effects observed, including in the organs
- Administered cutaneously, percutaneous absorption (LD50 in mg/l)
 - ◆ Effects observed. including in the organs
- Skin irritation
 - ◆ The substance should be applied to the shaved skin of an animal, preferably an albino rabbit.
 - ◆ Duration of exposure in hours.
- Eye irritation
 - ◆ The rabbit is the preferred animal
 - ◆ Duration of exposure in hours
- Skin sensitization
 - ◆ To be determined by a recognized method using a guinea pig
- Sub-acute toxicity (28 days)
 - ◆ Effects observed on the animal and organs according to the concentrations used, including clinical and laboratory investigations
 - ◆ Dose for which no toxic effect is observed
 - ◆ A period of daily administration (five to seven days per week) for at least four weeks should be chosen. The route of administration should be the most appropriate having regard to the intended use, the acute toxicity and the physical and chemical properties of the substance. Unless there are contra-indications, the rat is the preferred species for oral and inhalation experiments.
- Mutagenicity (including carcinogenic pre-screening test)
 - ◆ The substance should be examined during a series of two tests one of which should be bacteriological. with and without metabolic activation. and one non-bacteriological.

ECO-TOXICOLOGICAL STUDIES

- Effects on organisms
- Acute toxicity for fish (LC50 in ppm)
 - ◆ Duration of exposure
 - ◆ Species selected (one or more)
- Acute toxicity for Daphnia (LC50 in ppm)
 - ◆ Duration of exposure
- Degradation - biotic and abiotic
- The Biological Oxygen Demand and the Biological Oxygen Demand/Chemical Oxygen Demand ratio should be determined as a minimum.

POSSIBILITY OF RENDERING THE SUBSTANCE HARMLESS

- For industry/skilled trades
 - ◆ Possibility of recovery
 - ◆ Possibility of neutralization
 - ◆ Possibility of destruction:
 - controlled discharge
 - incineration
 - water purification station
 - others.
- For the public at large
 - ◆ Possibility of recovery
 - ◆ Possibility of neutralization
 - ◆ Possibility of destruction:
 - controlled discharge
 - incineration.
 - water purification station
 - others.

1.5.4. Material Safety Data Sheets

Much of the information above can be incorporated into the standardized *Material Safety Data Sheet*. The MSDS is a complete, up-to-date set of information for each product that a company manufactures or uses which, by law, must be available at all times in the Health or Safety office. The US Occupational Health and Safety Agency requires MSDSs for at least 600 chemicals. Companies are not required to perform tests, but to *list what they know*, including:

T1.24. COMPONENT PARTS OF THE MATERIAL SAFETY DATA SHEETS

SECTION I. Identity

Product name, family/chemical name, product type, Department of Transport category, hazard rating, fire rating, reactivity, emergency phone response number, prepared by...

SECTION II. Hazardous Ingredients

Ingredients with ACGIH, OSHA TLVs PELs and CAS no. plus source of information. Flags the hazardous ingredients.

SECTION III. Physical Data

Physical state, specific gravity, density, boiling point, percent volatiles, evaporation rate, odor, appearance.

SECTION IV. Fire and Explosion Hazard

Flash point, flammable limits, autoignition temperature, extinguishing media, fire-fighting procedures.

SECTION V. Health Hazard Data

Primary route of exposure (dermal, oral, eye, inhalation), over-exposure effects (irritation, sensitization) for acute and chronic effects, special toxicity (mutagenicity, teratogenicity, carcinogenicity, reproduction/fertility). Emergency and first aid procedures.

SECTION VI. Reactivity Data

Stability, incompatibility (materials to avoid), conditions to avoid, hazardous decomposition products, hazardous polymerization.

SECTION VII. Spill or Leak procedures

Spill control: how to do it, and what with. Waste disposal methods.

SECTION VIII. Special protection information

Respiratory protection (type of equipment needed), protective clothing (gloves, suits, eye, shoes).

SECTION IX. Special precautions

Handling, shipping and storage precautions, special warnings.

SECTION X. Regulatory information.

- ✚ the ingredients and quantities in the formulation,
- ✚ the physicochemical properties of each ingredient,
- ✚ the safety procedures and emergency first aid treatments,
- ✚ a section (Section V) dealing with toxicity data.

A quick review of most MSDSs reveals rather limited toxicity information. Section V usually relates to adverse health effects from acute, high level exposure through the oral, ocular, cutaneous or respiratory pathways, usually expressed as LD₅₀ (in mg/kg body weight) as determined in a rodent model. An example of a rather rich MSDS, for benzene, is available at: [Principles of Toxicology DVD:\ Benzene MSDS.pdf](#).

The delayed effects are usually more sparingly documented, even if, for certain classes of chemicals (halogenated hydrocarbon solvents, pesticides), the delayed toxicity is of greater concern. Any longer term (subchronic) toxicity test is generally also in the form of a rodent study.

The MSDS database is essential because workers can be exposed accidentally to chemicals during manufacture, transportation, and ultimate use. Invariably, accidents will occur despite training, precautions, etc.

1.5.5. Poisoning Databases

Acute toxicity studies can go far beyond the determination of the LD₅₀, for example into the measures needed to manage acute poisonings in humans.

The first emergency centers were created in 1949 in Copenhagen and Budapest. Information became systematized and computerized more thoroughly in the 1950s, when centers aimed at pediatric and general population poisonings started to grow. Most were operated as part of the emergency or

pharmacy of a hospital, with little training and no full time staff. There has been a tendency since the 1970s to reduce the number of poison centers and to provide them with better trained staff. Children under the age of 5 are responsible for 60 % of calls to poison centers.

The most used databases for poison centers are POISINDEX, DRUGDEX, TOMES and TOXNET.

For example, TOXNET is a computer system run by the National Library of Medicine that includes a number of toxicological databases managed by the Environmental Protection Agency, the National Cancer Institute, and the National Institute for Occupational Safety and Health. For more specific information, go to <http://www.toxnet.nlm.nih.gov>

The databases included in TOXNET are:
 CCRIS (Chemical Carcinogenesis Research Information System),
 DART (Developmental and Reproductive Toxicity Database),
 DBIR (Directory of Biotechnology Information Resources),
 EMICBACK (Environmental Mutagen Information Center Backfile),
 GENE-TOX (Genetic Toxicology),
 HSDB (Hazardous Substances Data Bank),
 IRIS (Integrated Risk Information System, from EPA),
<http://www.epa.gov/iriswebp/iris/index.html>
 RTECS (Registry of Toxic Effects of Chemical Substances),
 and
 TRI (Toxic Chemical Release Inventory).

For example, HSDB contains chemical-specific information on:

- ✚ manufacturing and use,

- ✚ chemical and physical properties,
- ✚ safety and handling,
- ✚ toxicity and biomedical effects,
- ✚ pharmacology,
- ✚ environmental fate and exposure potential,
- ✚ exposure standards and regulations,
- ✚ monitoring and analysis methods,
- ✚ and additional references.

1.5.6. Hygiene and Research Databases

1. A booklet for hygiene and for general toxicology work, NIOSH's Pocket Guide to Chemical Hazards (2005) is a short toxicity summary for hygiene purposes (T1.25). A condensed version is provided at [Principles of Toxicology CD:\NIOSH Pocket Guide to Chemical Hazards 2007.pdf](#) or at <http://www.cdc.gov/niosh/npg/>.
2. For in-depth toxicity work, Sax's *Dangerous Properties of Industrial Materials* provides more information (1.26). It delivers in extremely abbreviated form the results of 45 toxicity studies for benzene, a real time-saver when doing research work.
3. The Internet. See [Principles of Toxicology DVD:\Web Pages\WWW Risk Assessment Resources.pdf](#). Anyone wishing information about an agent has access to Internet resources, and may take the time to read basic research articles.

As a toxicologist, you will be called upon to interpret this database for your employers, the employees, those considering litigation against a company, etc.

Benzene											
Synonyms & Trade Names Benzal, Phenyl hydride											
CAS No. 71-43-2	RTECS No. CY1400000			DOT ID & Guide 1114 130 P							
Formula C ₆ H ₆	Conversion 1 ppm = 3.19 mg/m ³			IDLH Ca [500 ppm]	See: 71432						
Exposure Limits											
NIOSH REL : Ca TWA 0.1 ppm ST 1 ppm See Appendix A OSHA PEL : [1910.1028] TWA 1 ppm ST 5 ppm See Appendix F											
Physical Description Colorless to light-yellow liquid with an aromatic odor. [Note: A solid below 42°F.]											
MW: 78.1	BP: 176°F	FRZ: 42°F	Sol: 0.07%	VP: 75 mmHg	IP: 9.24 eV						
Sp.Gr: 0.88	FLP: 12°F	UEL: 7.8%	LEL: 1.2%								
Class IB Flammable Liquid: FL.P. below 73°F and BP at or above 100°F.											
Incompatibilities & Reactivities Strong oxidizers, many fluorides & perchlorates, nitric acid											
Exposure Routes inhalation, skin absorption, ingestion, skin and/or eye contact											
Symptoms irritation eyes, skin, nose, respiratory system; dizziness; headache, nausea, staggered gait; anorexia, lassitude (weakness, exhaustion); dermatitis; bone marrow depression; [potential occupational carcinogen]											
Target Organs Eyes, skin, respiratory system, blood, central nervous system, bone marrow											
Cancer Site [leukemia]											
Personal Protection/Sanitation (See protection codes)				First Aid (See procedures)							
Skin: Prevent skin contact	Eye: Irrigate immediately			Skin: Soap wash immediately							
Eyes: Prevent eye contact	Breathing: Respiratory support			Breathing: Medical attention immediately							
Wash skin: When contaminated	Swallow: Medical attention immediately										
Remove: When wet (flammable)											
Change: No recommendation											
Provide: Eyewash, Quick drench											
Respirator Recommendations (See Appendix E)											
NIOSH											
At concentrations above the NIOSH REL, or where there is no REL, at any detectable concentration: (APF = 10,000) Any self-contained breathing apparatus that has a full facepiece and is operated in a pressure-demand or other positive-pressure mode (APF = 10,000) Any supplied-air respirator that has a full facepiece and is operated in a pressure-demand or other positive-pressure mode in combination with an auxiliary self-contained positive-pressure breathing apparatus											
Escape: (APF = 50) Any air-purifying, full-facepiece respirator (gas mask) with a chin-style, front- or back-mounted organic vapor canister Any appropriate escape-type, self-contained breathing apparatus											
Important additional information about respirator selection											
See also: INTRODUCTION See ICSC CARD: 0015 See MEDICAL TESTS: 0022											

T1.25. NIOSH's Pocket Guide to Chemical Hazards.

BENZENECAS RN: 71432
mf: C₆H₆; mw: 78.12

Clear colorless liquid. mp: 5.51°, bp: 80.093°-80.094°, flash p: 12°F (CC), d: 0.8794 @ 20°, autoign. temp.: 1044°F, lel: 1.4%, vol: 8.0%, vap. press: 100 mm @ 26.1°, vap. d: 2.77, ucl: 95-100.

SYNS:(6)ANNULENE
BENZEN (DUTCH)
BENZEN (POLISH)
BENZOL
BENZOLENE
BENZOLO (ITALIAN)
BICARBURET OF HYDROGEN
CARBON OIL

NIOSH #: CY 1400000

COAL NAPHTHA
CYCLOHEXATRIENE
FENZEN (CZECH)
MINERAL NAPHTHA
MOTOR BENZOL
NCI-C55276
PHENYL HYDRIDE
PYROBENZOLE**TOXICITY DATA:**

skin-rbt 15 mg/24H open MLD	CODEN: AIHAAP 23,95,62
eye-rbt 88 mg MOD	AMIHAB 14,387,56
eye-rbt 2 mg/24H SEV	28ZPAK .23,72
cry-rat-scu 12 gm/kg/12D-I	GTPZAB 17(3).24,73
rat-mus-ipr 500 µL/kg	ENMUDM 2,43,80
cyt-mus-orl 100 µL/kg	ENMUDM 2,43,80
cyt-mus-ipr 100 µL/kg	ENMUDM 2,43,80
dit-mus-ipr 5 mg/kg	TPKVAL 15,30,79
cyt-rbt-scu 8400 mg/kg	PSDTAP 15,275,74
scu-mus TDLo: 2700 mg/kg/(13D prep): TER	AMBNAS 17,285,70
ihl-hmn TC:100 ppm/10Y-1:CAR	TRBMAV 23,95,62
ori-rat TDLo:52 gm/kg/52W-1:CAR	MELAAD 70,352,79
skin-mus TDLo:1200 gm/kg/ 49W-1:NEO	BJCAAI 16,275,62
scu-mus TDLo:600 mg/kg/ 17W-1:ETA	KRANA9 9,403,32
par-mus TDLo:670 mg/kg/ 19W-1:ETA	KLWOAZ 12,109,33
ihl-hmn TC:400 ppm/8Y-1:ETA	BLOOAW 52,285,78
ihl-mas TC:1200 mg/m3/4Y-1:CAR	NEJMAC 271,376,24
ori-rat TD:10 gm/kg/52W-1:CAR	MELAAD 70,352,79
ori-hmn TDLo:130 mg/kg/CNS	AHYGAJ 31,336,1897
ihl-hmn LCLo:20000 ppm/5M	29ZUA8 ..,53
ihl-hmn TCLo:210 ppm:BLD	27ZXA3 ..,341,63
ihl-rat TCLo:670 mg/m3/24H (15D prep): TER	HYSAAV 33,327,68
ihl-rat TCLo:56600 ug/m3/24H (1-2D prep)	HYSAAV 33,112,68
ihl-rat TCLo:50 ppm/24H (7-14D prep)	JHEMA2 24,363,80
ihl-rat TCLo:150 ppm/24H (7-14D prep)	JHEMA2 24,363,80
scu-mus TDLo:1100 mg/kg (12D prep)	TOXID9 1,125,81
scu-mus TDLo:2700 mg/kg/(13D prep) TFX: TER	AMBNAS 17,285,70
ori-mus TDLo:9 gm/kg (6-15D prep)	TJADAB 19,41A,79
ori-mus TDLo:12 gm/kg (6-15D prep)	TJADAB 19,41A,79
ori-rat TD:10 gm/kg/52W-1 TFX: CAR	MELAAD 70,352,79
ihl-hmn TCLo:100 ppm:CNS	INMEAF 17,199,48
uhk-mas LDLo:194 mg/kg	85DCAI 2,73,70
uhk-mas LDLo:3800 mg/kg	TXAPAK 19,699,71
ihl-rat LC50:10000 ppm/7H	28ZRAQ ..,113,60
ipr-rat LDLo:1150 mg/kg	TXAPAK 1,156,39
ori-mus LDLo:4700 mg/kg	HYSAAV 32,349,67
ihl-mus LC50:9980 ppm	JHATAB 25,366,43
ipr-mus LDLo:990 ug/kg	AGGHAR 18,109,66
ori-dog LDLo:2000 mg/kg	HBMAMK 4,131,35
ihl-dog LCLo:146000 mg/m3	HBTXAC 1,324,56
ihl-cat LCLo:170000 mg/m3	HBTXAC 1,324,56
ivn-rbt LDLo:88 mg/kg	JTEHD6 (Suppl 2),45,77
ipr-gpg LDLo:527 mg/kg	HBTXAC 1,42,56
scu-fog LDLo:1400 mg/kg	HBMAMK 4,131,35
ihl-mam LCLo:20000 ppm/5M	AEPPAE 138,65,28

BENZENE 361Aquatic Toxicity Rating: TLm96:100-10 ppm WQCHM*
2,-74. Carcinogenic Determination: Human Suspected
IARC** 7,203,74.

TLV: Air: 10 ppm DTLVS* 4,37,80. *Toxicology Review:* ARPAAQ 11,434,31; EVHPAZ 11,163,75; AEHLAU 22,373,71; PAREAQ 4,1,52; FNSCA6 2,67,73; MU-REAV 47(2),75,78; AMSVAZ 118,354,44; ZHPMAT 166,113,78; JTEHD6 -(suppl.2),69,77; PHRPA6 41,1357,26; CTOXAO 11,531,77; BYNYMAN 54, 413,78; KRANAW 9,403,32; 27ZTAP 3,22,69. OSHA Standard: Air: TWA 10 ppm; CL 25 ppm; PK 50 ppm/10M/8H (SCP-U) FEREAC 39,23540,74. DOT: Flammable Liquid. Label: Flammable Liquid FEREAC 41,57018,76. Occupational Exposure to Benzene recm std: Air: CL 10 ppm/60M NTIS**. Currently Tested by NTP for Carcinogenesis by Standard Bioassay Protocol as of December 1980. "NIOSH Manual of Analytical Methods" VOL 1 127, VOL 3 S311. Reported in EPA TSCA Inventory, 1980. EPA TSCA 8E NO:12770027-Followup Sent as of April, 1979.

THR: Poisoning occurs most commonly through inhal of the vapor, though benzene can penetrate the skin, and poison in that way. Locally, benzene has a comparatively strong irr effect, producing erythema and burning, and, in more severe cases, edema and even blistering. Exposure to high conc of the vapor (3000 ppm or higher) may result from failure of equipment or spillage. Such exposure, while rare in industry, may result in acute poisoning, characterized by the narcotic action of benzene on the CNS. The anesthetic action of benzene is similar to that of other anesthetic gases, consisting of a preliminary stage of excitation followed by depression and, if exposure is continued, death through respiratory failure. The chronic, rather than the acute form, of benzene poisoning is important in industry. It is a recog leukemogen. There is no specific blood picture occurring in cases of chronic benzol poisoning. The bone marrow may be hypoplastic, normal, or hyperplastic, the changes reflected in the peripheral blood. Anemia, leucopenia, macrocytosis, reticulocytosis, thrombocytopenia, high color index, and prolonged bleeding time may be present. Cases of myeloid leukemia have been reported. For the supervision of the worker, repeated blood examinations are necessary, including hemoglobin determinations, white and red cell counts and differential smears. Where a worker shows a progressive drop in either red or white cells, or where the white count remains below 5,000 per cu mm or the red count below 4.0 million per cu mm, on two successive monthly examinations, he should be immediately removed from exposure. Following absorption of benzene, elimination is chiefly through the lungs, when fresh air is breathed. The portion that is absorbed is oxidized, and the oxidation products are combined with sulfuric and glycuronic acids and eliminated in the urine. This may be used as a diagnostic sign. Benzene has a definite cumulative action, and exposure to relatively high conc is not serious from the point of view of causing damage to the blood-forming system, provided the exposure is not repeated. On the other hand,

T1.26. Sax's Dangerous Properties of Industrial Materials.

1.6. Toxicologists in Society

What do toxicologists do ?

Some are employed by the government (Health Canada), others by the private sector. They can be involved in the protection of workers (Occupational Health) or of the general population (Public Health).

The may be concerned with pharmaceuticals (clinical toxicologists, poison specialists), household products, industrial and agricultural chemicals and food additives.

They perform tests in laboratories (research and development, evaluation of test results), risk analysis (literature reviews) and develop policy and regulations for government or corporations.

They may serve on committees determining Safe Human Doses or product safety and labeling regulations.

Environmental toxicologists are concerned with the persistence of chemicals in the environment, food chain accumulation and with the consequences of toxicants to the biota.

They may be involved in public relations, in scientific writing for the public and in legal proceedings, as expert witnesses.

The Society of Toxicology lists the following specialties:

- Biological Modeling,
- Carcinogenesis,
- Comparative and Veterinary toxicology,
- Dermal toxicology,
- Food Safety,
- Immunotoxicology,
- In Vitro toxicology,

- Inhalation toxicology,
- Mechanistic toxicology,
- Metals toxicology,
- Molecular toxicology,
- Neurotoxicology,
- Epidemiology,
- Occupational Health,
- Regulatory and Safety evaluation,
- Reproductive and Developmental toxicology,
- Risk Assessment,
- Toxicological and Exploratory pathology...

Of course, toxicologists may also pursue academic careers in universities.

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2. Risk Assessment

2.1. Complexity of Toxic Effects

All toxic agents have multiple effects occurring at different doses, and within specific time intervals, as shown in F2.1. A critical decision in the practice of Risk Assessment is focusing on one or a few toxicity variables to estimate the Safe Human Dose. Which critical toxicity variable or disease rate is most affected by the agent, and to what extent should exposure be limited to protect human or animal health?

Extensive data for a particular toxicity variable or test system may be available simply because a laboratory was funded to conduct the research, or because the measurement is convenient or inexpensive (ex, Ames test). The toxicologist aims to compensate for these biases, looking to obtain from restricted evidence a toxicity determination that is as comprehensive as possible.

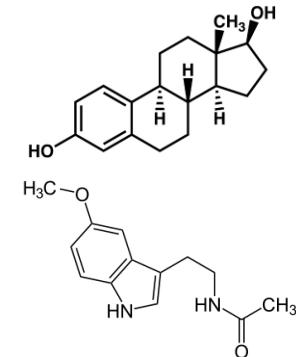
2.1.1. Low-Dose Toxic Effects

The appreciation of the full toxicity of an agent often proceeds historically through a series of successive discoveries. For example, it has been known for a long time that arsenic kills rapidly in high doses (8 mg/kg in the rat).

But it is relatively recent knowledge that at doses of 7 ppm in water, it leads to narrowing of the carotid artery¹ and that it can act as an endocrine disruptor^{3,5}. Further, it interferes with the action of glucocorticoids at an environmental concentration of 10 ppb.

More subtle toxic effects are often uncovered over time. Environmental hormones, compounds that can alter physiology

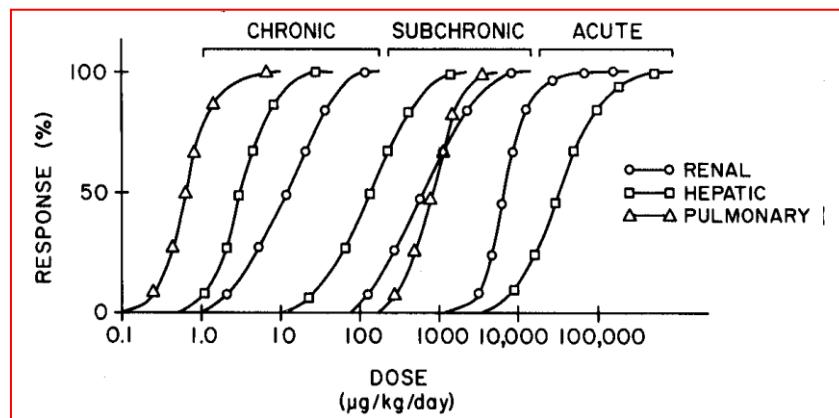
at low concentrations, are prime candidates for low-dose risks. Five years ago, almost all cases of environmental hormones (ex, nonylphenol, phthalates⁶) involved estrogen (estradiol is shown). More recently, agents substituting melatonin (shown), anti-androgens and thyroid hormones have emerged.



2.1.2. Significant vs Trivial Risks

Protective limits for an agent should take into account its lowest-dose *significant* risk. The decision of which risk to retain as *significant* is one that must be made on the basis of the deepest knowledge of physiology and epidemiology available.

Perhaps the greatest weakness of Risk Assessment is that its view is limited by the evidence available. It is impractical to test *all* body systems in sufficient detail to dispel *all* doubts about the safety of an agent.



F2.1 Plurality of Effects for a Toxicant.
In Vitro Methods in Toxicology, Jolles and Cordier, 1990

Files compiled for an agent typically contain a large number of studies. A first triage is usually made on the basis of

- ✚ species (human, rodent, cells),
- ✚ route of administration (inhaled, cutaneous, oral exposures) and
- ✚ time-frame (acute, chronic).

If biological exposure indices such as blood or plasma levels are available, this helps to make comparisons between studies more enlightening, particularly if the targets of the toxicant are known.

An important practical problem is that Safe Human Doses are intended to limit lifetime exposures, while few laboratory studies ever provide this time frame. Epidemiology often provides a more chronic perspective than experiments, but typically its results are much more difficult to interpret.

2.1.3. Tox File Diversity

The diversity of scientific knowledge can become an obstacle to obtaining a clear assessment of risk. Some studies often promote particular interests and methods, and high political stakes often tend to disintegrate a unified picture of risk. This can give the impression of an absence of coherence or reliability in science. The real problem is that risk is itself fairly complex in that it can apply to different organs, populations and situations. It is therefore often difficult to come to a single limit that will satisfy all points of view. Often the Safe Human Dose will be determined by focusing on a few *strategically chosen* studies.

2.2. The Choice of Graphs

The choice of graphic representations for toxicity data as a function of dose is important because graphs have a suggestive influence. One frequently quoted motive to use *logarithmic* axes is to view more clearly data spanning a very wide

amplitude range (orders of magnitude). On a linear scale, the relative changes occurring at low values would be obscured, compressed in the early part of the graph.

F2.2 shows the same financial data (Dow Jones industrial average) presented in 3 different ways. One gets quite a different impression about the evolution of the economy, according to the axial metric chosen...



F2.2. The leftmost graph (linear-linear) gives the impression of a rapidly expanding economy in recent years. The middle graph (log – linear) suggests a steady evolution. The rightmost graph (log-log) shows a tendency to saturation. So, how is the economy *actually* evolving? *American Scientist*, 89:210

Note the following about log scales.

- There is no “0”.
- A log scale displays normal number labels that are positioned according to a log scale. The grids along the log scale change abruptly from intervals of 10 to intervals of 100, for example.
- You cannot re-number these labels, but you can choose the position of the decimal points.
- Equal-sized steps along a log scale correspond to a multiplication factor.

Toxicology uses specific conventions in its choice of graph axes, and these conventions can be justified using Michaelis-Menten kinetics or statistical arguments, as we shall see later in this chapter. The graphic conventions (log axes) used in Toxicology are set to ease detection of specific dependencies anticipated from experimental results. However, one should use log scales cautiously...

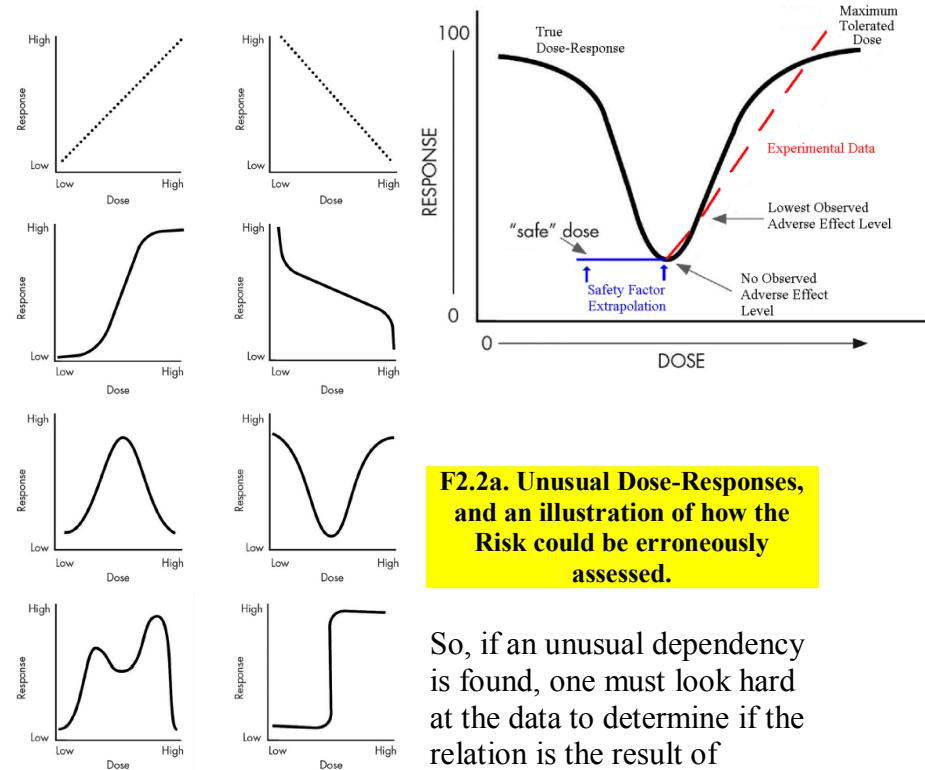
Do not let tricky graphs cloud your thinking about problems they are intended to illuminate.

2.3. Dose-Response Relations

Quantitative Toxicology relates exposure (usually expressed as toxicant concentration x exposure duration) and the toxicity produced (changes in liver, renal, neurological, pulmonary function, etc.), in other words, the dose-response relationship.

There is a great deal of emphasis in Toxicology on documenting fairly conventional, progressive dose-response relationships similar to F2.3. Obtaining a “proper” relation gives the experimenter or the epidemiologist confidence that his data is sound. However, this frequently observed curve type is not the only one possible, as was shown by the class of chemical known as “endocrine disruptors”¹⁰, for example, and one should be prepared to accept unusual dose-response dependencies. Agents can be more toxic at lower doses than at higher ones, as can be seen in T2.2a and F2.2a. Examples include pollutants that resemble hormones, drugs⁴, electromagnetic radiation and ionizing radiation.

T2.2a. Unusual Dose-Effect Curves		
Agent	Observation	Ref.
di-2-ethylhexyl phthalate (DEHP, a plastic-softening agent)	Low DEHP dose suppresses brain activity of aromatase, critical for male development, while higher doses stimulate it.	9
X-rays and Gamma radiation	1 Gy increases lifetime risk of cancer by 5 percent, while 0.1 Gy defends against cancer.	8
Electromagnetic Radiation	Calcium efflux in nerve cells is stimulated within an intensity window only.	7



F2.2a. Unusual Dose-Responses, and an illustration of how the Risk could be erroneously assessed.

So, if an unusual dependency is found, one must look hard at the data to determine if the relation is the result of artefacts or of true

physiology. Unusual dose-responses are less likely in more complex systems. Therefore, epidemiologists scrutinizing large populations are less likely to observe them than laboratory experimenters.

Emphasis on documentation of the dose-response tends to obscure other aspects of toxicity, specifically:

- ✚ whether the outcome variable chosen is the most relevant one to determine a Safe Human Dose for the agent,
- ✚ the influence of time: most tests are too short (to conserve resources), thereby blinding the testing process to the

evolution and reversibility of toxicity.

"**DOSE**" refers to the mass administered (ex, 10 mg of diazepam) and does not account for the size or weight of the animal. Generally, *body burden* is also expressed as an absolute mass of substance.

"**DOSAGE**" refers to the mass per body weight of the individual (ex, 0.2 mg/kg of body weight).

"**TOTAL DOSAGE**" takes into account the duration of exposure: a dosage of 10 mg/kg per day for 10 days translates to a Total Dosage of 100 mg .day / kg.

2.4. Characteristics of Dose- Response Curves

Many dose-response curves can be characterized according to the following characteristics: Efficacy, Potency, Spread and Statistical Dispersion.

2.4.1. Efficacy

The curve between dosage and resulting toxicity defines whether a given agent (say, caffeine) is *effective* at producing a given toxicity (say, a rise in heart rate).

The *efficacy* of an agent relates to the maximum effect that it can produce at any concentration (see F2.8).

Often, it is also appropriate to express the *efficacy* of an agent by comparing it to the maximum effect that can be obtained on a living system by the most effective chemical known. The ratio of the particular agent's result to the system's maximum response thus determines *efficacy* in a range from 0 to 1.

2.4.2. Potency

An agent's efficacy tells us something about its maximum effect, but not about how much of the agent is needed to obtain a given effect. A "reading" of how much of the agent (mg,

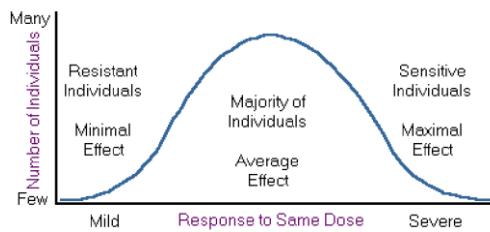
concentration or total dosage) is required to obtain half of the maximum effect (efficacy = 0.5) is commonly used to derive a measure of toxicant *potency*^Ø.

A drug that requires a lower dose than another drug to obtain the same half-maximum effect is therefore considered to be more *potent*. F2.7 illustrates 3 curves of different potency.

2.4.3. Spread

Some dose-response curves are very steep, while others need larger changes of dosage for the same change in toxic effect. F2.4 shows curves with different *spread*. *Spread* can be specified by the toxicant dosage ratio between 10 % and 90 % efficacy. In F2.4, the curve on the left has a 10-90 % *spread* of 21.9, while the curve on the right has a 10-90 % *spread* of 3.88.

2.4.4. Statistical Dispersion



If a group of animals in a laboratory study is given exactly the same dose of a chemical, the response from each animal will be at least slightly different.

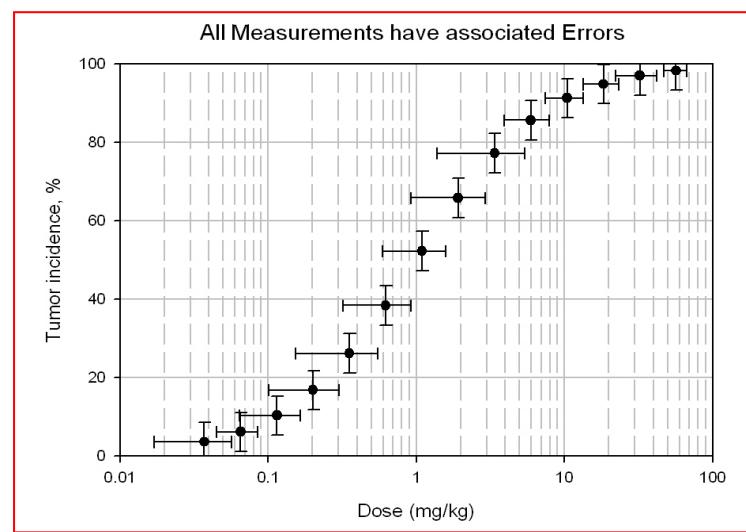
There is also an uncertainty as to the dose actually received by each animal. For example, in the example above, the toxicant may be mixed with food, and each animal does not eat exactly the same amount. In laboratory studies, the inaccuracy in the

^Ø Drug expiration dates specify the manufacturer's guarantee of *full potency* and safety. **It is safe** to take drugs past their expiration dates. Even 10 years past the date, most drugs have a good deal of their original potency. So, if your life depends on an expired drug and you *must* have 100 % of its original strength, you should toss it, but if not, use it and see what happens. 90 % of drugs are safe and effective as far as 15 years past their expiration dates. Exceptions: nitroglycerin, insulin and some liquid antibiotics.

dose (horizontal) is usually fairly small, but the dispersion of the toxic reaction (vertically) cannot be controlled. For example, if one uses a group of 6 animals, yielding 6 individual toxicity responses at a given dose, these 6 outcomes can be used to determine a mean response, as well as a standard deviation for this outcome at that dose.

Even in the case where the outcome is rated as all-or-nothing, such as presence of cancer, the number of animals used in any given study limits the reliability of the probability of getting cancer, particularly if the risk is small.

Although laboratory experimenters mostly attempt to reduce the dispersion in their data by keeping tight control over animals (to the point of using animal clones) and test conditions, *dispersions in toxicity reactions are part of the data*, if the data is to be relevant to a complex environment. In epidemiological studies, there are uncertainties associated with gathering data from populations, and also uncertainties in the estimates of their exposures.



F2.3. Horizontal and Vertical dispersions.

In any dose-response graph, such as F2.3, both the variable describing the toxicity (here, tumor incidence), as well as that describing dose (horizontal), show statistical dispersions. Therefore, obtaining data dispersions representative of real-world situations may be legitimate objectives in laboratory experiments as well as in epidemiological investigations.

2.5. Analysis of Dose-Response Curves

2.5.1. Multi-Target Model

We study toxic dose-responses by building simple mathematical models of toxicity. Let us imagine that we initially have a group of N_0 cells exposed to a toxic dose (D). A number of cells (N) survive the exposure, while the others die. If we perform the experiment repeatedly at various doses, we can obtain dose responses such as in F2.4.

Considering the set of experiments on the blue curve, the supplementary number of cells dying (dN) per exposure increment (dD) is proportional to the number of cells (N) living at the lower dose multiplied by the “toxic slope” variable, “ t ”. This assumes the same toxic slope at all levels of toxicity.

$$-\frac{dN}{dD} = t \ N$$

After exposure “ D ”, the cell number declines from N_0 to N :

$$\int_{N_0}^N \frac{dN}{N} = -t \int_0^D dD$$

$$\ln \frac{N}{N_0} = -t D$$

The proportion of surviving cells is:

$$\frac{N}{N_0} = e^{-t D}$$

In this simple model, an infinite dose is needed to kill all the cells.

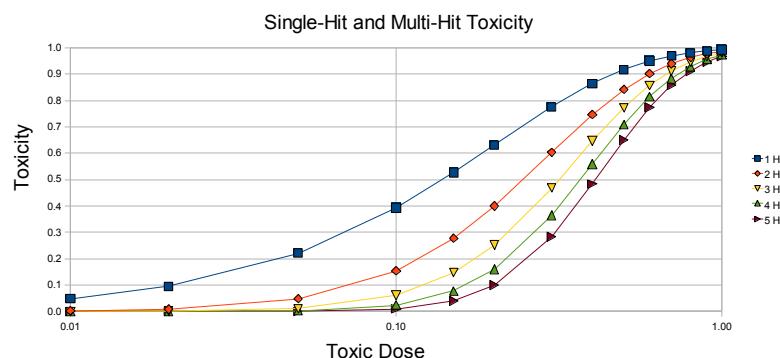
We can define Toxicity (T) as the fraction of dead cells:

$$\text{Toxicity} = \frac{No - N}{No} = \frac{No - No e^{-tD}}{No} = 1 - e^{-tD}$$

At this point, we modify the model slightly. The death of a cell can occur from a single toxic hit (F2.4, blue curve), or it can occur from numerous “hits” (below) to a single cell. The probability that n independent events of probability P occur is P^n . In this Multi-Hit model, the probability of death decreases, because “n” random hits must accumulate for one cell’s death:

$$\text{Toxicity} = (1 - e^{-tD})^n$$

A semi-log graph of Toxic Dose (D) vs Toxicity is drawn for $n = 1$ to 5 hits in F2.4.



F2.4. Multi-Hits make dose-responses steeper.

The Single-Hit curve (blue) displays the condition “ $-dN/dD = tN$ ”, while the higher n -curves tend toward a threshold form. Threshold dose-responses are frequently observed in Toxicology. The 5-Hit curve’s shape results from the fact that 1 to 4 hits do not produce toxicity in the left part of the curve, while in the right part, there is a relative richness of vulnerable

targets (with accumulated hits), coupled with high toxicant concentration. The multiple hit curves are functionally equivalent to a low-dose detoxification mechanism. Note the 10-90 % spread in these curves.

T2.4a. 10-90 % Toxicity Spread					
1-Hit	2-Hit	3-Hit	4-Hit	5-Hit	10-Hit
21.9	7.82	5.4	4.42	3.88	1.91

When is the Single-Hit model applicable ?

The plant toxin ricin follows a retrograde transport route: from cell surface to endosomes to the Golgi complex, to the endoplasmic reticulum and finally the cytosol, where it binds to and inactivates ribosomal RNA, thereby inhibiting protein synthesis. A single ricin molecule entering the cytosol can inactivate over 1,500 ribosomes per minute, and kill the cell.

When is the Multi-Hit model applicable ?

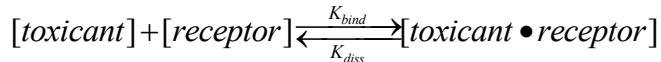
The inactivation of membrane receptors is an example, because they are usually available in larger number than necessary. Toxicity emerges at the toxicant concentration where the remaining active receptor proteins are *required* for cell survival or function.

If absorbed in small enough quantities, most toxicants can be neutralized by normal physiological mechanisms such as Phase II metabolism (Chapter 3). This neutralization would shift the dose-response curves to the right.

2.5.2. Toxicant to Receptor Binding

Increasing the stimulation on a physiological system generally produces climbing curves that saturate at higher doses. *Equilibrium binding* can be applied to toxicant-receptor kinetics.

When *toxicants* interact with biological *receptors*, binding follows the *law of mass action*. The equation below describes the reversible binding of *toxicant* to *receptor*, quantified by binding and dissociation constants (K_x).



The rate of bound complex (•) formation in moles/minute = $[\text{toxicant}] \times [\text{receptor}] \times K_{\text{bind}}$. Consequently, the binding rate constant K_{bind} is expressed in units of 1/(moles x min).

The rate of complex dissociation (+) is $[\text{toxicant} \bullet \text{receptor}] \times K_{\text{diss}}$. Consequently, the dissociation rate constant K_{diss} is expressed in units of 1/min.

At equilibrium, the dissociation reaction equals the binding reaction, so

$$[\text{toxicant}] \times [\text{receptor}] \times K_{\text{bind}} = [\text{toxicant} \bullet \text{receptor}] \times K_{\text{diss}}$$

or

$$[\text{toxicant} \bullet \text{receptor}] = [\text{toxicant}] \times [\text{receptor}] \times \frac{K_{\text{bind}}}{K_{\text{diss}}}$$

Define the Michaelis-Menten constant, K_M , equal to $K_{\text{diss}}/K_{\text{bind}}$, in moles units, which yields:

$$[\text{toxicant} \bullet \text{receptor}] = \frac{[\text{toxicant}] \times [\text{receptor}]}{K_M}$$

Low values of K_M indicate that the complex holds together very tightly. Since all *receptors* are either free or bound, we can express $[\text{receptor}]$ as the total number of receptors minus the number bound:

$$[\text{receptor}] = [\text{receptor}_{\text{tot}}] - [\text{toxicant} \bullet \text{receptor}].$$

Substituting this definition into the previous equation,

$$[\text{toxicant} \bullet \text{receptor}] = \frac{[\text{toxicant}] \times ([\text{receptor}_{\text{tot}}] - [\text{toxicant} \bullet \text{receptor}])}{K_M}$$

which can be rearranged into the *binding equation* as:

$$[\text{toxicant} \bullet \text{receptor}] = \frac{[\text{receptor}_{\text{tot}}] \times [\text{toxicant}]}{K_M + [\text{toxicant}]}$$

In toxicity testing, we vary the dose (or *toxicant* concentration) and measure toxic effects proportional to $[\text{toxicant} \bullet \text{receptor}]$, the left side of the equation above. Both $[\text{receptor}_{\text{tot}}]$ and K_M are constants.

F2.5, created from the preceding equation, represents a *binding curve*. If you plot the same data using a semilog layout (the X-axis is *log of dose*), it becomes a soft sigmoid curve, as shown in F2.6.

The 10-90 % spread in these curves is about 100, independent of K_M .

This curve is the expected form of toxicity relationships between log of dose and continuous toxicity variables, if compatible with simple receptor-ligand kinetics.

This new *binding curve* has a central range where the slope changes relatively little, one practical reason for adoption of these axes. An estimate of the amount of the toxicant that produces a 50 % response is obtainable from the data points that contribute to the estimation of this central slope. Deviations from this form signal that more complex phenomena contribute to toxicity.

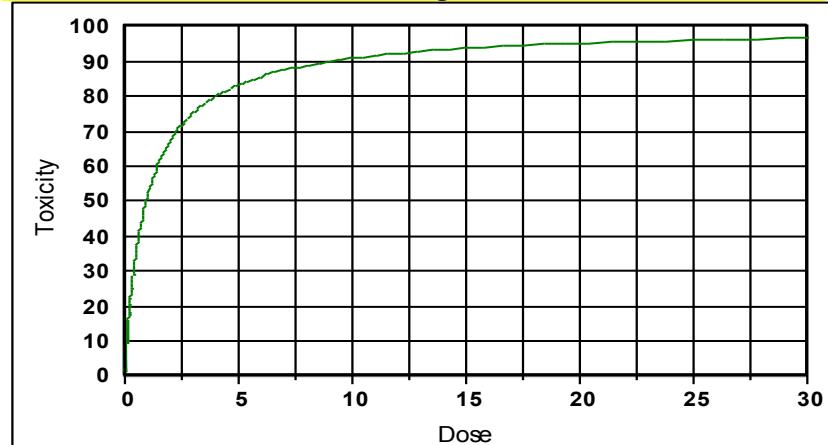
Further, if we change the value of the Michaelis-Menten constant (K_M) in the binding equation by a factor of 100 each way, identically shaped toxicity curves (F2.7) display variations in *potency*. These curves could be perceived as showing a threshold or not, depending on the range of dose chosen for the experiment.

The value of the Michaelis-Menten constant, K_M , represents the speed of chemical reactions in general, and is known for several enzymes (T2.4b).

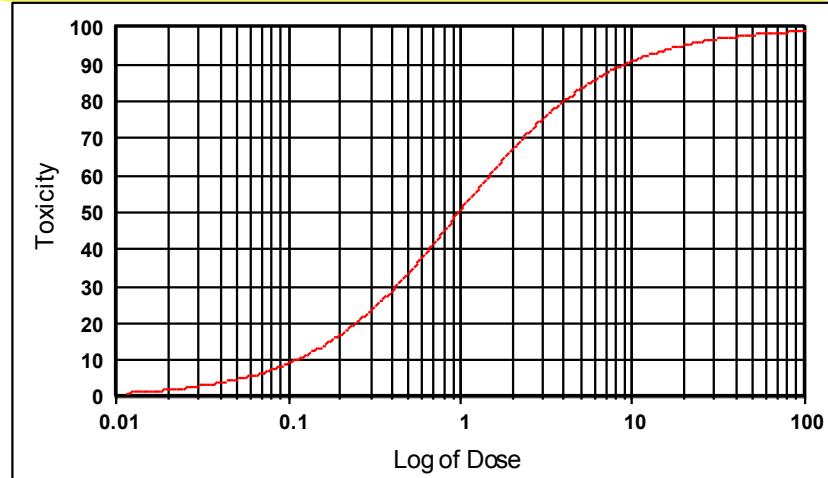
If we wish to represent analytically changes in efficacy, the term “+[toxicant]” in the binding equation can be modified by addition of a factor corresponding to inclusion of two binding steps as opposed to one, the two successive steps cascading to contribute to a final expression of toxicity. The result is illustrated in the lower curve of F2.8, showing that two binding steps reduce the efficacy of the reaction.

T2.4b. Michaelis-Menten constants, Km		
Enzyme	Substrate	K _m (Molar)
Catalase	H ₂ O ₂	1.1
Chymotrypsin	Gly-Tyr-Gly	0.108
Carbonic anhydrase	CO ₂	0.012
β -Galactosidase	d-Lactose	.004
Acetylcholinesterase	Acetylcholine (ACh)	0.00009
β -Lactamase	Benzylpenicillin	0.00002

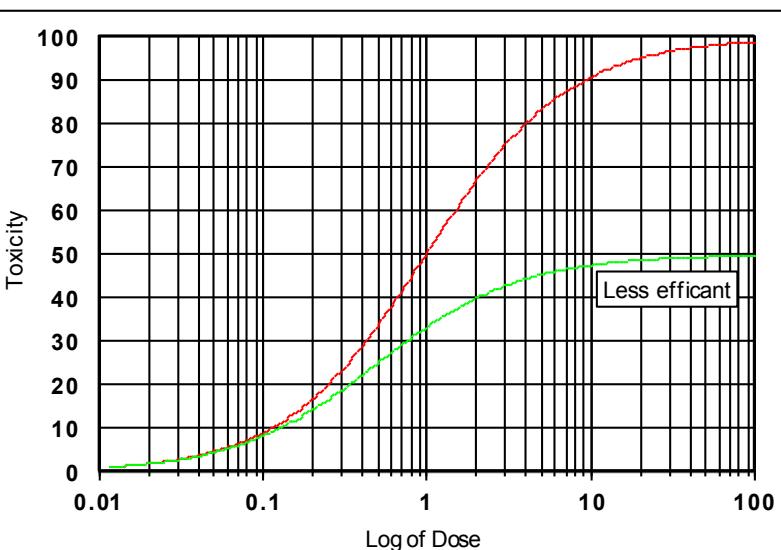
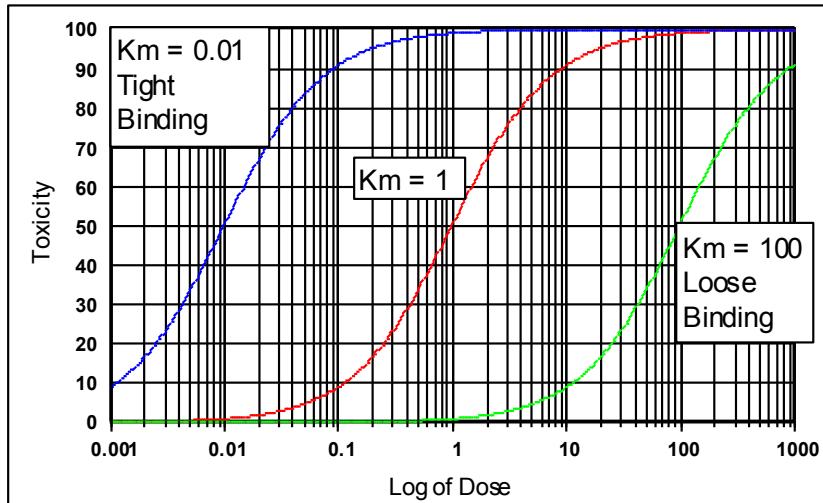
F2.5. Binding Curve.



F2.6. Log scaling of a Binding Curve.



F2.7. Changing K_m alters Toxic Potency.



F2.8. Increasing number of binding steps reduces Efficacy.

2.5.3. Dose-Response of Complex Systems

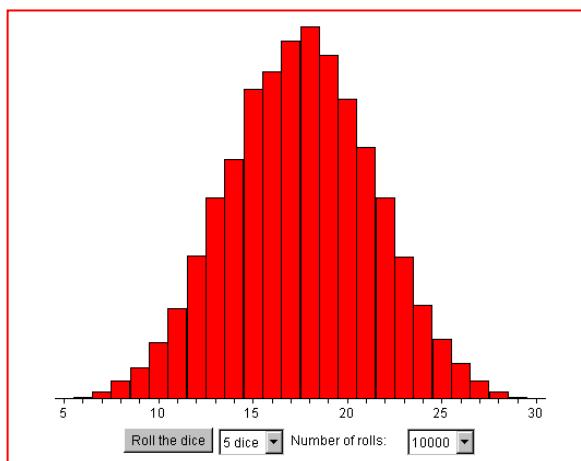
In laboratory tests, the toxicologist almost always prefers to evaluate groups of animals or cells, basically because all animals or cells do not behave identically. In epidemiology, differences between human subjects lead to the determination of probabilities, as opposed to certainties.

To protect toxicological results against individual variations, a statistically significant sample of animals or cells is needed for testing. This plurality of test objects is almost universally implemented, often according to budget limitations, even if no *a priori* information is available on the variability of the toxicity investigated.

2.5.3.1. Central Limit Theorem

To view how test-subject variations influence toxicological results, we take a brief tour of simple statistics...

If one rolls one die repeatedly, the values 1,2,3,4,5,6 will come out the same number of times, resulting in a flat histogram.



F2.9. Distribution generated by rolling 5 dice.

<http://www.stat.sc.edu/~west/javahtml/CLT.html>

However, as the number of dice increases, the distribution of the total results assumes the shape in F2.9. Even if the distribution for the individual die is flat, the sum of the readings of 5 dice will show a most frequent value (18) between the extremes (5 and 30), because there are fewer solutions for extreme values of the roll (low=1,1,1,1,1 or high=6,6,6,6,6) than there are for intermediate values.

Each of the 5 dice represents a variable which can have 6 toxic sensitivity levels (1,2,3,4,5,6). All 5 variables (dice) contribute to the subject's overall toxicity dose-response (total score).

In order to obtain *extreme* values of toxicity sensitivity, either low or high, all 5 variables must contribute *extreme* characteristics, while more moderate thresholds can be obtained using *many more possible combinations of the individual variables*. The *normal distribution* that applies to the dies (F2.9) also applies to integrated toxicity, if a sufficiently large number of factors determine toxicity outcome.

If integrated toxicity is *normally distributed* with respect to dose, few animals exhibit toxicity at extreme doses, while many show toxicity at intermediate values (dose of 500, as shown by the red line in F2.10).

If we are interested in the proportion of all animals that have reached the toxic threshold at a given dose, we integrate the red-line distribution (Gaussian) into the green curve (F2.10).

Further, if we graph the two previous curves over a log abscissa, we obtain the *Gaussian response* graph in F2.11.

We note here that the green curve shows, as in the example of the toxicant-receptor model (F2.6), a soft sigmoid curve with a central range where the slope changes relatively little.

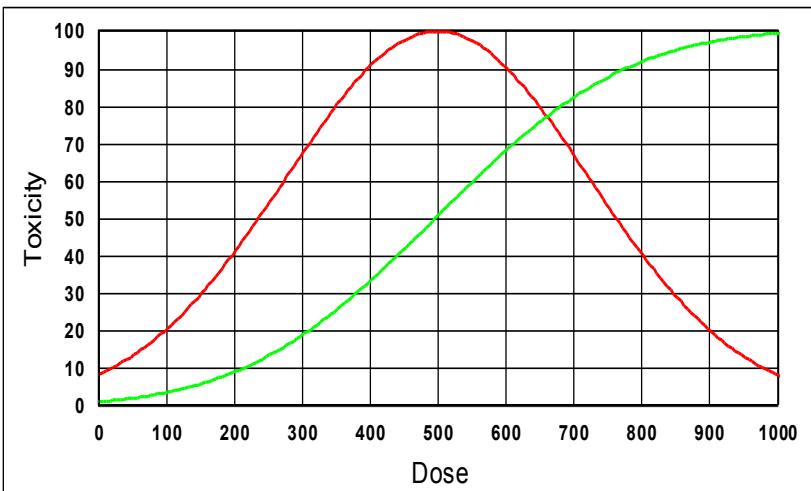
The *Central Tendency Theorem* says that if we average more and more independent random quantities that have a common distribution (and if that common distribution is not too *pathological*), then the distribution of their means approaches a Gaussian.

Departures from this Gaussian (normal) probability distribution suggests that special variables are strong determinants of toxicity.

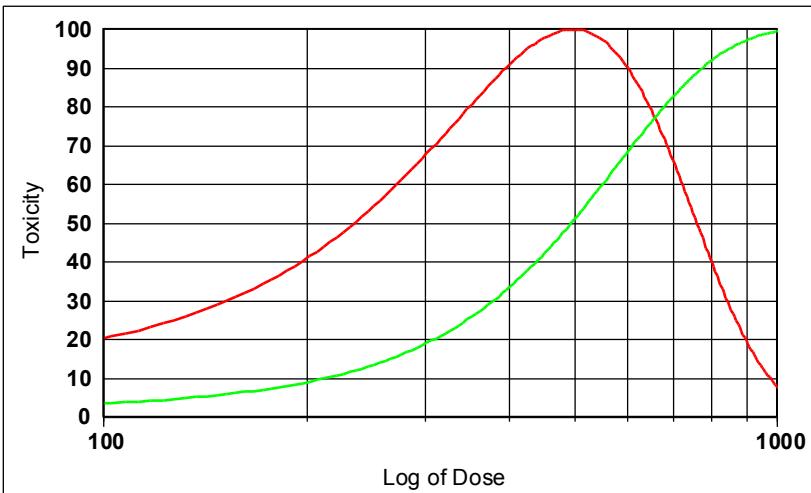
The variability within toxicant targets widens the spread of dose-response curves with respect to those obtainable from the simplest experimental systems.

Therefore, the physiology lab working on simple systems should display narrower spreads than those observed in epidemiology, or in groups of animals.

However, whether dealing with a continuous variable or threshold expression of toxicity in a group, in the physiology lab or in epidemiology, dose-responses are likely to display similar shapes over a log scale.



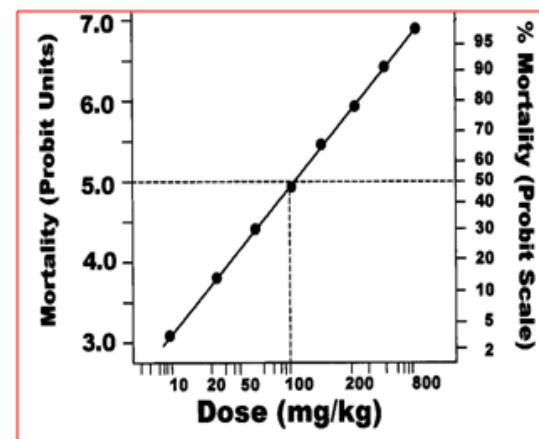
F2.10. Gaussian toxicity distribution.



F2.11. Log scaling of Gaussian toxicity distribution.

2.5.3.2. Probit Scale

An effort has been made to straighten completely the curve obtained from randomly determined (Gaussian) toxicity dependencies over dose. In this procedure, the proportion of subjects responding to each dose is transformed using a computed table into a number called a *probit*. The table is built assuming that the experimental events are distributed exactly according to a Gaussian. The graph of probit against log of dose often produces points that are linear over a wide region of dose in actual experiments (F2.12). The ideal probit plot is a straight line, with a slope equal to $1/\sigma$.



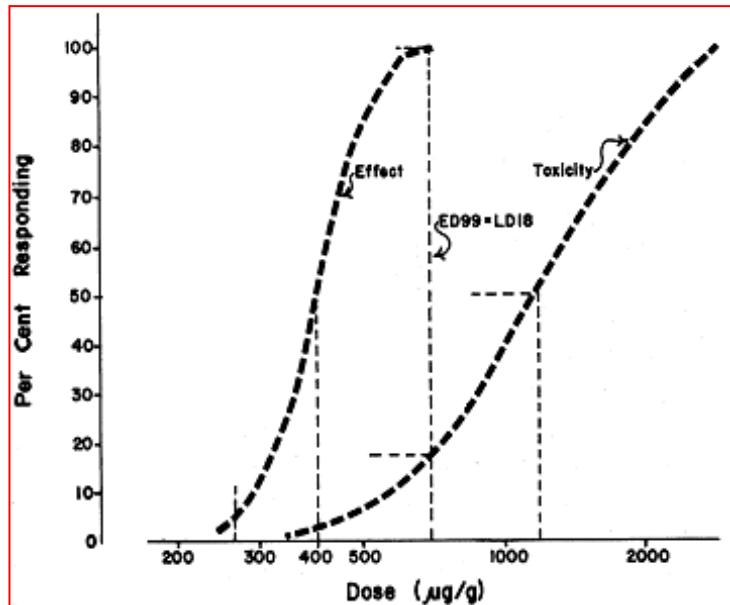
F2.12. A probit curve.

The processes of linearization used in toxicology allow for easier quantitative assessment of experimental results, and also facilitate the detection of extraneous contributions within simple results, as deviations from a straight line.

2.6. Dose-Response Examples

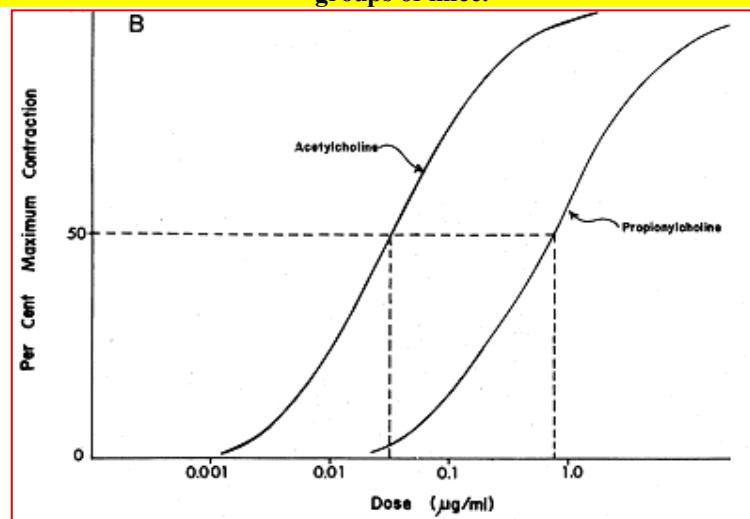
2.6.1. Example 1: Rat Ileum

F2.13 shows continuous dose-responses with administration of various concentrations of two drugs in the guinea pig ileum. These curves have a shape similar to the *binding curves* displayed previously. The 10-90 % spreads are 79 and 100.



F2.13. Acetylcholine and propionylcholine in the rat ileum.

F2.14. Loss of righting reflex (left) and cessation of breathing (right) in groups of mice.



2.6.2. Example 2: Cancer in Rats

One common task in toxicology is to assess what proportion of test animals will show a response at various strengths of exposure. In the determination of lethal doses, there are only two outcomes: either the animal dies, or it lives (a *quantal* response). Similarly, in cancer studies, either there is a tumor or there is not. Cumulative curves similar to the ones in F2.13 can be documented for rat carcinogenicity.

2.6.3. Example 3: Anesthesia

Studying loss of righting reflex ("Effect") and cessation of breathing ("Toxicity") in groups of 20 mice yielded the graphs in F2.14. These curves are similar to the *Gaussian response* curves displayed previously. The 10-90 % spreads are 1.8 and 4.

As might be expected, a number of parameters can be adjusted within the mathematical expressions of the theoretical dose-response characteristics displayed previously, to fit experimental data. Observations cover the full range of 10-90 % spreads discussed theoretically in the earlier sections.

2.7. Hazard & Risk Assessment

2.7.1. Components of Risk Assessment

Some definitions...

The "**HAZARD**" produced by an agent is the injury that will occur as a result of exposure.

A "**RISK**" combines the **hazard** with **exposure**, yielding a **probability** that the injury will occur. For example, the probability of dying in an aircraft crash (hazard) is one per 8 million flights (risk).

Risk Assessment is made of components often occurring in succession.

Hazard Identification: the identification of known or potential health effects associated with a particular agent.

Hazard Quantification (Toxicology): the quantitative evaluation of the hazards associated with an agent. The aim is to collect all relevant human, animal and *in vitro* studies of toxicity.

Abstracted databases such as Sax's *Dangerous Properties of Industrial Materials* can be invaluable in such work. From this database, for each species, relationships between the dosage administered (mg/kg/day, mg total dose, etc.) and the effects produced can be developed in order to establish:

- + the animal species or *in vitro* system most representative of humans for the *endpoints of toxicity retained*, and
- + the dosage range and the shape (linear, convex, concave) of the dose-response relationship.

If the dosage range used in animal studies is different from that encountered in exposed humans, there is need to extrapolate to the human situation.

Exposure Quantification (Hygiene): determination, hopefully by actual measurement, of the amount of the toxicant to which individuals may be exposed. This will include air, water, soil, food analysis, the nature and size of potentially exposed populations, the duration of exposure (usually in the past), the routes by which the agent might be acquired.

Risk Setting: One of the issues in risk assessment is how *acceptable* risk levels are decided. There are standardized levels of risk that attempt to balance the acceptable risk with a particular situation.

A **risk of 1 per Million** is considered acceptable for bystanders and volunteers. This low risk is attributed because they are considered to be unsuspecting and naïve potential victims.

A **risk of 1 in 100,000** is considered acceptable for general patients receiving drugs, since they are expected to get a benefit from the drugs they are using.

A **risk of 1 in 10,000** is considered acceptable in workplace safety, since the subjects earn a salary, are likely aware of the exposure, and make the production of all goods possible.

A **risk of 1 in 1,000** is considered acceptable for patients that are victims of life-threatening diseases, since drug administration may represent their only chance for survival.

Risk Quantification: integration of hazard and exposure quantification into an estimation of the adverse effects likely to occur in a given population, including data uncertainties. A critical aspect is whether the dose-response relationship is:

- + threshold: there is a level below which toxicity (in animals or humans) does not occur, or
- + non-threshold: there is always some toxicity, regardless of how small the exposure is.

It is not uncommon that risks associated with certain agents need to be updated due to new evidence or new analysis of old evidence. One of the most powerful methods of manipulating risk quantification is by expert assessment of publications, establishing the relative credibility of specific studies (expert assessment can be biased).

For example, in 2007, the Occupational Safety and Health Administration (OSHA) amended the occupational standard of

exposure to hexavalent chromium (Cr(VI)) because workers faced a significant risk of lung cancer, asthma, and damage to the nasal epithelia and skin at the current exposure limits. The new rule establishes an 8-hour time-weighted average (TWA) exposure limit of 5 $\mu\text{g}/\text{m}^3$ of Cr(VI). The previous limit was 100 $\mu\text{g}/\text{m}^3$ (a division by a factor of 20).

The new standard regulates separately general industry, construction, and shipyards in order to tailor requirements to the unique circumstances found in each of these sectors. The new standard reduces the risk posed to workers to the maximum extent that is technologically and economically feasible.

Legal exposure limits reduce risks to levels that are considered acceptable by the standards of the time.

For example, the old standard meant that a worker with a 45-year maximum exposure to Cr(VI) had a cancer risk between 10 and 35 % (added to all other cancer risks), while the new standard is expected to induce cancers at rates of 1 to 4.5 %

Risk Decision: The weighing of policy alternatives to accept, minimize or reduce risks, and to select and implement appropriate options. Risk decision may involve, for example, implementation of protective measures within an industrial plant. If you are a member of a national committee, it may involve settling for a value of the Threshold Limit Value. For the Environmental Protection Agency (EPA) and the International Agency for Research on Cancer (IARC), who assess the carcinogenic potential of agents based on the "weight of evidence"¹ approach, risk decision is the assignment of an agent to a specific class (see Chapter 13).

Risk Communication: Issues often occur in relation to dissemination of toxicity information, or use of biased information. If there is conflict, such as in a court case, all background data is usually shared between parties as documents are tabled. From the same information, the parties then develop their interpretation, and attempt to sway the tribunal in their favor.

If the controversy gets into the media, and it will, if it is contentious, your interpretation of the potential hazard should be presented rationally and convincingly.

Next page: F2.15. Types of Evidence used in Risk Assessment.

¹ This is a decidedly, and perhaps deliberately, vague concept.

TYPES OF EVIDENCE USED IN RISK ASSESSMENT

The diagram illustrates the increasing significance of evidence from left to right, represented by a yellow double-headed arrow. The top part of the arrow is labeled "INCREASING SIGNIFICANCE TO PHYSIOLOGY (MECHANISMS)" and the bottom part is labeled "INCREASING SIGNIFICANCE TO HUMAN HEALTH".

	Humans (<i>in populo</i>)	Animals (<i>in vivo</i>)	Higher Cells (<i>in vitro</i>)	Lower Cells (<i>in vitro</i>)	Molecular Biology (<i>in vitro, in silico</i>)
METHOD	Cross-sectional or longitudinal studies of prevalence or incidence.	Particularly rodents, because of their low cost.	Human or mammalian cells and tissues.	Lower unicellulars, such as bacteria.	Molecular experiments or computer simulations.
EXPOSURE	Sampled or estimated in epi., approx. controlled in clinical studies.	Approximately controlled.	Well controlled.	Well controlled.	Tightly controlled or defined.
TOXICITY	Human diseases.	Tumors, biopsies.	From cell function.	From cell function.	Inferred.
TIME FRAME	From acute poisonings up to lifelong (80 yrs for cancer incidence).	Up to lifelong (2 yrs) or even longer in reproductive studies.	Up to a week.	Days.	Hours.
TYPICAL TESTS	Epidemiology and Clinical studies.	Carcinogenicity study in rats.	Cytotoxicity (survival and proliferation of cells).	Ames test for genotoxicity.	Structure-Activity Relationships.

2.7.2. "Strength of Evidence" Criteria

To confirm that *associations* are *causal* between agent tested and the effect found, we use "**Strength of Evidence**" criteria.

Time: toxicity indexes should grow after exposure starts (latency) and (generally) fall after exposure stops, in a time-frame compatible with toxicant elimination, or recovery from toxicity.

Strength of toxic reaction: a strong toxic effect is less likely than a weak one to be due to baseline noise in an animal model or to uncontrolled factors (that also unwittingly influence the toxic reaction studied). Therefore, a stronger reaction is less likely to be artefactual than a smaller one.

Dose-response: finding a threshold, as well as a simple dose-response, increases the chance that the data describes a toxic reaction. While it is not necessary that the dose-response follows a classic curve, data points along the dose curve should support, rather than discredit each other.

Consistency: toxicities are most convincing when they can be observed across species (despeciation), and when they can be confirmed using many laboratory techniques or toxicity indicators.

Plausibility: is there basic molecular knowledge that can explain the toxicity data observed ? Is an alternate explanation for the observations possible ?

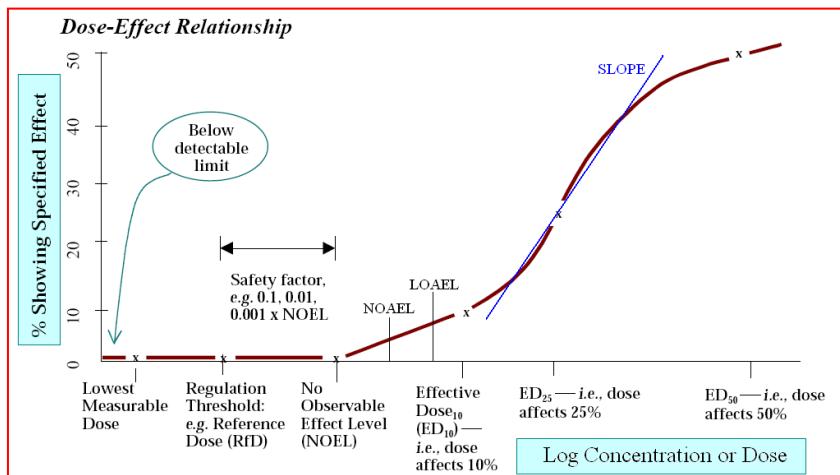
Compatibility: are the new toxicity observations compatible with existing knowledge, or do they leave us with an incompatibility with the established databases ?

2.7.3. Threshold-Relationships

Most of the chemical-induced toxicities you will encounter fall in the classification of "threshold relationships". In those toxicities, the detoxification term in Haber's Law is not zero.

This means that below some concentration of exposure, no toxic effect will be detected or detectable.

Having developed a dose-response relationship for an endpoint of toxicity in the most susceptible species, what useful quantities might be recovered from the relationship (F2.16)?



F2.16. Dose-response relationship with the parameters that may be recovered from the curve: NOEL, NOAEL, LOAEL, ED₅₀, Slope, FEL.

- + **FEL:** the frank effect level, where most of the individuals demonstrate the anticipated toxic effect.
- + **ED₅₀:** as in the acute toxicity studies, where the LD₅₀ was determined for lethality, this value gives the dose hazardous to 50 % of the individuals. In cancer, this means induction of tumors in 50 % of animals.
- + **SLOPE** of the dose-response relationship, indicating whether a change in dosage produces a small or large alteration in toxic effect.
- + **LOAEL:** Lowest Observable Adverse Effect Level, a dosage that still causes some adverse health effect, frequently being the lowest dosage administered in a 3-dose experiment.

- ✚ **NOAEL:** the No Observed Adverse Effect Level, the dosage that may cause some changes in status (reduction in body weight, organ function, etc.) but which are *not considered detrimental to general wellbeing*. In many cases, this may also be the lowest dosage administered in a 3-dose experiment.
- ✚ **NOEL:** the no observed effect level - a dosage administered that has *no measurable effect* on the test individuals, the subjects looking and behaving (both physiologically and biochemically) exactly like the controls. This parameter is not easily obtained from studies, the lowest dosage given usually producing a NOAEL or LOAEL.

2.7.4. Safety Factors

Under the assumption of a threshold relationship for toxicity, a *low-toxicity level* is quantified from experiments, yielding one or more of the above parameters: NOEL, NOAEL or LOAEL, in decreasing order of preference.

The *low-toxicity level* is then divided by a *Safety Factor*, often chosen in multiples of 10. For example, a Safe Human Dose may result from dividing the NOEL by a safety factor of 100. Such a *Safety Factor* takes into consideration:

- ✚ that the human may be up to 10-fold more sensitive than the animal species in which the study was done (inter-species difference), and
- ✚ that there may be a 10-fold range in sensitivity between the very young, the adult and the elderly within any human population (intra-species difference).

If a NOEL cannot be obtained from the dose-response relationship, then the assessor may work with a NOAEL or

LOAEL, and may increase the magnitude of the *Safety Factor* from 100- to 1000-fold.

This addresses the problem that the threshold observed represents uncertain toxicity.

One may also use fractions, say 250 or 500 fold, depending upon your assessment of the quality of the information available. With any *Safety Factor* greater than 1000-fold, one begins to suspect a flimsy database.

The result of the computation represents a *Safe Human Dose*, an acceptable level of exposure or concentration via the normal human absorption route.

Safe Human Doses are also labeled:

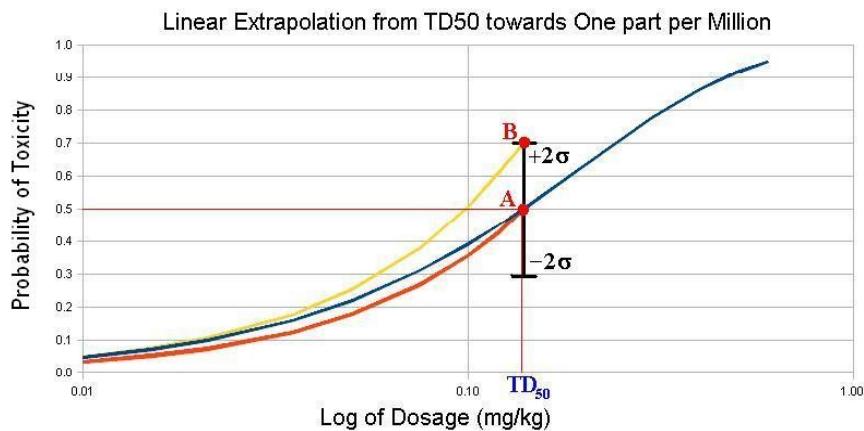
- ✚ maximum allowable concentration (MAC),
- ✚ reference dose (RfD),
- ✚ virtually safe dose (VSD),
- ✚ estimated population threshold for humans (EPT-H),
- ✚ acceptable daily intake (ADI), or
- ✚ tolerable daily intake (TDI).

A committee of the National Academies of Science pored over 50 years of data on survivors and descendants of survivors of the Hiroshima and Nagasaki atomic bomb attacks, as well as accumulated data from dozens of studies on exposures to workers in the nuclear industry and other high-exposure sites. Their conclusion is that any exposure to natural or man-made radiation, even at a very low level, carries a cancer risk. In the past, the nuclear industry and some independent experts have suggested that a safe low threshold for radiation exposure exists. Even at the highest end of what experts consider low-dose lifetime exposure, 100 millisieverts, about 1 person in 100 would develop cancer. To put that number in context, 42 other

lifetime cases of cancer can be expected in that same population of 100, due to such factors as smoking, environmental toxins or other influences. The impact of such conclusions related to one of the most tightly documented agents, ionizing radiation, could have impacts on Toxicology as a whole.

2.7.5. Non-Threshold Relationships

When dealing with carcinogenicity, where one molecule of an *ultimate carcinogen* could produce a cancer (Single-Hit), it is often assumed that the dose-response relation is *non-threshold*. This means that there is residual risk as long as there is *any* exposure.



F2.17. Linear extrapolation methods from 50 % probability to 1/1,000,000 probability. From A, a line is drawn starting at the mean value of TD₅₀ towards a probability of 1/1,000,000. From B, a line is drawn starting at the upper 95 % confidence limit of TD₅₀. A linear extrapolation does not show as a straight line on a linear-log graph, and there is no zero on the "LOG. DOSAGE" scale. Extrapolations could also be made from the TD₁₀ levels.

In F2.17, one chooses a Threshold Dose point (point A) on the dose-response curve (TD₅₀), and “linearly” extrapolates

towards zero, to some conventional level of risk, such as 1/1,000,000 (or 1/100,000).

A more protective estimate can be obtained if one uses the upper limit (+2 standard deviations) of the TD₅₀ (point B). The +2σ value can be statistically determined from the experimental data.

Assessors typically use two or more of these starting points, obtaining multiple scenarios, and finally choosing among them later on.

The use of linear models for the assessment of carcinogen hazards arose from early work on radiation, where effects on cells were *expected* to relate linearly to particle emissions detected by Geiger counters (1940's - 1950's). This concept was transferred *in toto* to mutagenic chemicals, which did not emit anything, but could be shown to interact with nucleic acids at relatively low concentrations.

Arguments rage even today around whether or not there is a threshold level for carcinogens, but analysis has demonstrated a non-linear, Gaussian (sigmoid-shaped) relationship between radiation and biological effects.

It is easy to draw straight lines, but the exact relation between dose and effect at low doses is usually a matter of speculation, because of the lack of reliable experimental results. People and assessors naturally get very nervous when they suspect that they might be underestimating risks. To this end, complicated and sophisticated models based on a variety of toxicological assumptions have been developed.

The level retained as the reference point for risk calculation (A or B) is then extrapolated to an *acceptable risk of cancer*, arbitrarily established usually as 1 case/1,000,000 or 1 case/100,000 of population. Such a level would go undetected by routine epidemiology, eliminating the scenario where human deaths could be proven from anything but the most sophisticated examination of health files. Furthermore,

experiments to reliably prove such low risks would be prohibitively expensive.

If, for example, incidence of cancer rose by 10 % at a dose of 1 mg/kg/day, the Safe Human Dose would be 100 ng/kg/day, assuming extrapolation to a risk of 1/100,000.

There has been a recent trend in the USA in particular to unify cancer risk assessment with other risk assessment techniques, resulting in a Unified Safe Human Dose approach for carcinogens *and* non-carcinogens.

The proposed assessment may start from a NOAEL and apply to this level 10x Safety Factors to a maximum of 10,000.

Beyond the normal Safety Factors classically applied, such as:

- ✚ need to extrapolate from animal tests to humans, and
- ✚ existence of sensitive individuals,

the following factors are commonly considered:

- ✚ exposure of children (Reproductive Toxicology),
- ✚ limited availability of chronic data, and
- ✚ any special “modifying factors”.

For cancer data, a 10 % excess of disease above baseline determines the “Benchmark Dose” or "Effective Dose" at 10 % (ED_{10}). The Safe Human Dose for the carcinogen would be obtained as $LED_{10} / 10,000$ or other safety factor. “L” in LED_{10} refers to a lower statistical limit (1 sigma) of the Effective Dose.

2.7.6. Integration of Multiple Exposures

Philosophical differences between risk assessment methods become apparent when integrating effects of multiple agents.

The *non-threshold* method, commonly applied to carcinogens, provides a simple method of integration. The basic observation in a non-threshold model is, for example, that 1 mg/day of agent “A” produces a lifetime cancer rate of 1 in a million.

Environmental Limits vs Occupational Limits

The U.S. EPA, in seeking to protect general populations from carcinogens, tolerates cancer rates of 1/1,000,000 to 1/10,000 for most of their guidelines. The U.S. Occupational Safety and Health Administration, in accomodating economic feasibility, puts workers at much higher risk than EPA, as shown in the Table below (highest EPA risk is 0.1/1000 workers).

Excess Cancers per 1000 workers at OSHA Permissible Exposure Level	
Ethylene Oxide	1.2-2.3
Asbestos	6.7
Benzene	10
Formaldehyde	0.0056-2.64
Methylenedianiline	0.8
Cadmium	3-15
1,3 Butadiene	1.3-8.1
Methylene Chloride	3.6
Chromium VI	10.45

Most OSHA limits are to some extent obsolete, because of time-consuming committee work, leading to inevitable discussions in court.

This is a slope of 1/1,000,000 cancer-day/mg. If agent “B” is added with a similar risk, the cancer rates can simply be added to 2/1,000,000 cancer-day/mg:

$$\text{Integrated Risk} = \sum_i D_i \times \text{Slope of Carcinogenicity Curve}_i$$

For *threshold* risk assessment, the integration is more vague because the concepts relate to the “Safe Dose”, as opposed to a specific disease rate. When combining the effects of two or more agents, we obtain a compounded “Risk Ratio”, which sums the ratios between actual doses of agents (D_i) and doses considered “safe”.

$$\text{Risk Ratio} = \sum_i \frac{D_i}{\text{Safe Dose}_i}$$

“Safe” is an absolute and elusive concept, not a measured disease rate.

Both of the integrations above assume no interactions between the individual agents.

2.7.7. Precautionary Principle

A major difficulty in risk management is the public’s unwillingness to accept a low (estimated “safe”) level of carcinogens in air, water, food, etc. At one extreme, the public requires zero risk, an impossibility in this day and age, when our analytical capabilities can measure minute (ppt, ppq) amounts of contaminants. In the public’s psychology, if the agent has a concentration number, it should not be there, no matter how small the number may be.

Such an attitude is difficult to reconcile with technological feasibility. However, the notion that risks attendant to various agents are discovered by science *after* exposure has occurred is very strong in the public’s eye, and partly justified. The *final* experiment is that of human exposure...

Various philosophies are used in risk management, but one that has gained favor, particularly in the European Union (it is already law in Germany and Sweden), is the *precautionary principle*, also known as *prudent avoidance*.

“When an activity raises threats of harm to human health or the environment, precautionary measures should be taken even if some cause and effect relationships are not fully established scientifically.”

In effect, the Precautionary Principle shifts the burden of proof away from the protection advocates and towards industry. It also dictates the approach when evidence is lacking or is inconclusive.

The Precautionary Principle might have effects soon in influencing the inclusion of new chemicals on the UN’s list of Persistent Organic Pollutants (POPs). The list presently includes 12 pollutants: dioxins and furans, PCBs, aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, hexachlorobenzene, mirex and toxaphene.

2.7.8. Hazards of Risk Assessment

2.7.8.1. Toxicology vs Risk Assessment

The procedures of toxicology, with standardized tests and hopefully careful inferences, are perceived as more scientific than the deliberations of Risk Assessment. Hazard & Risk Assessment⁴ is a semi-scientific process usually involving many segments of society, and leading to decisions.

2.7.8.2. Objectivity in Risk Assessment

Weaknesses inherent in Risk Assessment techniques, for example

- (1) dealing with incomplete data,
- (2) focussing on specific sets of data,
- (3) the choice of Safety Factors and statistical methods,
- (4) relying on status quo (previous decisions or traditions),

underlie its vulnerability to politics, making exposure to toxicants a matter of *choice*.

1 - The lack of information creates opportunity for individuals to devise models, even quantitative models, that are susceptible to political influence.

As incomplete science meets a demand for decisions, small segments of science can be given too much importance.

All scientific work is incomplete-whether it be observational or experimental. All scientific work is liable to be upset or modified by advancing knowledge. That does not confer upon us a freedom to ignore the knowledge we already have, or to postpone the action that it appears to demand at a given time.

(Bradford Hill)

2 - Toxicity evidence may sometimes appear incoherent. Restoring coherence can be done by arbitrarily weighing the importance of data, giving more credibility to some, less to others, while still ignoring others entirely. The choices may depend on how hazards and risks are *perceived*.

Selection of scientific data can be used to support hidden economic and political agendas.

3 - The choice of quantitative technique, for example threshold vs non-threshold dose-response, can have a serious impact on the level of protection.

Statistics is an excellent servant and a bad master.

4 - Canada has viewed asbestos as a natural resource for decades, and is still exporting large amounts of asbestos to poor countries in Asia.

The recipients of profits have little incentive to objectively examine their products for adverse health consequences.

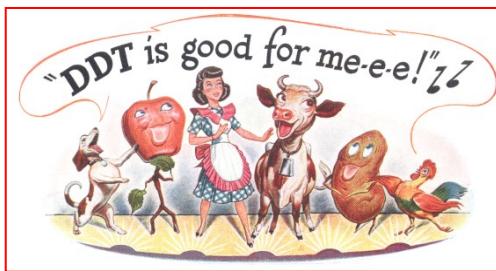
Many polemists represent these conflicts as a contest between financial interests (money) and human health.

Managers and politicians may place public interest behind self-interest

But “money” concerns are sometimes legitimate. Although we need to protect human health, the efforts that we marshal to protect it should not create more grief than the problems we are trying to solve. To strike a balance, objectivity is necessary, or perhaps more accurately, adoption of a point of view that balances the interests of everyone.

The cost of safety vs the desire for zero risk.

Gold standards of objectivity are hard to attain in difficult questions. In the distant past, objectivity was thought to issue from social position or high education. Today, the need for impartiality incites the use of more resources (committees) and technical means such as statistics (vs vague statements) and images (vs drawing or recounts) to enhance credibility.



Quantitative methods help, but they must be used adequately to produce the best results.

F2.18. From the June, 1947 issue of Time.

2.7.8.3. Example 1: DDT

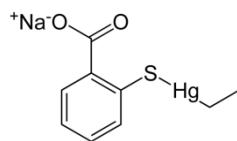
Dichloro-diphenyl-trichloro-ethane (DDT) was used extensively in the period from 1940 to 1960 to control insect populations. It was cheap, effective, persistent in the

environment and made a significant dent in the world incidence of malaria. It was applied in large quantities to cotton crops. But western public mood changed when unexpected environmental toxicities were found. Some concerned the fragilization of the eggs of wild birds, particularly the bald eagle, a sensitive subject in the US, where DDT was banned in 1971. Today, DDT is still surrounded in controversy, particularly because developing countries have not found inexpensive substitutes.

Whether DDT is acceptable could be decided using mountains of specific toxicity data. But the matter is even more easily settled depending on whether your country is currently infested by mosquitoes and malaria. There are at least 300 million acute cases of malaria each year, with more than a million deaths.

2.7.8.4. Example 2: Thimerosal

Thimerosal, which contains ethyl mercury, has been used to prevent bacterial contamination in vaccine vials over the last 50 years, apparently with a “good safety record”.



Work is going on to eliminate thimerosal from vaccines, in an effort to minimize exposure to mercury, particularly in children (see the document DVD:\Thimerosal Debate.pdf).

Thimerosal prevents the conversion of dietary forms of vitamin B₁₂ into the active form. This blocks methionine synthase, which interferes with DNA methylation (an epi-genetic control mechanism).

Whether thimerosal is acceptable depends on the availability of refrigeration for the conservation of vaccines, or on whether the cost of individual vials, as opposed to large multi-dose vials, is acceptable.

These two examples show that the use of a chemical usually has both positive and negative impacts. The balance between them can be compiled by a procedure of *Hazard and Risk Assessment*.

2.7.8.5. Factors Influencing Risk Assessment

The determination of how objectionable a given risk is depends on a number of factors, many intimately related to human fear:

- Is the injury heritable ?
- Is the injury permanent ?
- How debilitating is the injury ?
- Does a hypersensitive population exist ?

- Is the presence of risk recognized by the subjects (environmental vs occupational) ?
- Can exposure be avoided ?
- Are only a few individuals exposed, or is everyone ?
- Is the source natural or industrial ?
- Is the source controllable ?
- Is the source familiar ?
- Is the risk larger than other familiar risks ?
- Are there alternatives to the agent ?
- Is there a high potential for misuse ?

2.7.8.6. Manipulation of Risk Assessment

Biases in Risk assessment can result from improper scientific techniques. Here are a few examples.

- The investigative power of a study can be limited by using groups too small for the level of risk expected, insuring that the conclusions will have no statistical significance
- A study can be too short to see the full development of disease incidence.
- The selection subjects or the exposure systems can be poor so as to include non-exposed among the exposed, or

- incorporating large uncertainty margins in the exposure, weakening the potency of the study
- + Alter routes of exposure that do not affect targets as strongly as they should
- + Perform histology on samples not well preserved, so that effects of agents become undetectable

2.7.9. An Example of Risk Assessment

(modified from Williams & Burson)

This example sets a drinking-water standard for “chlorinated ethylene”, designating a group of compounds for which hypothetical but realistic data, largely derived from *perchloroethylene*, are used. It illustrates how various Safe Human Doses can be derived, according to different assumptions or data sources. The various SHD values (mg/day) obtained are highlighted in red. Emphasis was added to various parts of the text by your professor.

2.7.9.1. Introduction and Historical Perspectives

Chlorinated ethylene (CE), a volatile compound, is used as a degreaser for machinery and as a solvent for organic products. CE has been used in consumer products such as glues, rug cleaners, as a propellant for aerosol products, and as an extraction solvent for decaffeinated coffee prior to the late 1970s. Near that time, the Food and Drug Administration (FDA) prohibited its use as a direct or indirect food additive because of suspicions that CE was a carcinogen. Prior to the FDA ban, CE was measured in foods:

ITEM	ppm
Dairy products	1-10
Meat	10-20
Oils and fats	1-19
Hot beverages	160
Mollusks	0-0.250
Fish	0-0.48

Besides food sources, it was estimated that some 5000 medical, dental, and hospital personnel were exposed to CE and that about 60,000 additional persons were subjected to it annually when it was used as an anesthetic in medical situations. Concentrations in hospital rooms ranged from 0.3 to 103 ppm.

Based on its widespread use and occurrence in our environment, it is not surprising that levels of 1-32 ppb were reported post-mortem in human tissues.

The FDA therefore also moved against any medical uses and banned all anesthetics containing CE. Furthermore, CE was disallowed in cosmetic products, animal and pet foods, animal drug products, and as an oil-extraction solvent for seed products.

The Environmental Protection Agency (EPA) quickly followed the precedent of the FDA and issued notice that CE was a candidate for denial of renewed registration on the basis of its possible carcinogenicity.

The OSHA guidelines now limit air concentrations of CE in areas where employees work to **100 ppm** (500 mg/m^3) during an eight-hour time-weighted average period. The NIOSH (National Institute for Occupational Safety and Health) guidelines, however, recommend that no occupational exposure to halogenated anesthetic agents be greater than **2 ppm** (10 mg/m^3) for a one-hour sampling period.

2.7.9.2. Animal Metabolism and Kinetics

Absorption. CE is readily absorbed by all routes of exposure, as would be predicted by its physical and chemical properties. Its historical use as an anesthetic indicates its rapid rate and high percent of absorption via inhalation. Measurements taken after oral administration of CE to rats indicate that at least 80 percent is absorbed from the GI tract.

Elimination. The elimination of CE in mammals is fairly rapid. After inhalation of CE at 300 ppm for 8 hr, CE was undetectable in the expired air of rats in one study. However, another study indicated approximately 80 percent would be eliminated via the lungs. The estimated half-life for CE for most tissues is about 100 min, while for fat tissues the half-life is 200 min. Thus, eight hours after administration some 95 percent of the CE absorbed should be eliminated.

Metabolism. Much of the early toxicity research with CE was thought to support the idea that metabolism generated a reactive metabolite, probably the epoxide form, which was hepatotoxic and possibly capable of binding DNA and initiating cancer. However, recent work has changed much of the current thought on CE metabolism by demonstrating that the alcohol metabolite is not dependent upon nor derived from the epoxide metabolite form. This in turn suggests that species differences in hepatotoxicity and carcinogenicity are not related to species differences in the conversion rate of the epoxide to the less reactive alcohol metabolite. In addition, it has been shown that covalent protein binding of CE intermediates does not correlate with the formation of the epoxide metabolite. However, research has demonstrated that species differences do exist for covalent binding of reactive metabolites in rats and mice and that these differences in covalent binding parallel the different carcinogenic responses observed in these species.

Mechanistic Differences between Species. Interestingly, human microsomes form DNA adducts at a low rate similar to that found in the noncarcinogenic model, Osborne-Mendel rats. Both rats and humans appear to have substantially lower rates of microsomally generated DNA-adduct formation when compared to the B6C3F1 mouse, a species that is positive for liver cancer after chronic CE exposure. Researchers have also reported a species difference in microsomally generated covalent protein binding, which was likewise consistent with the results from the carcinogenicity tests. A sex difference in covalent binding in the B6C3F1 mouse has

also been demonstrated which is consistent with the higher liver cancer incidence observed in male mice.

Differences between Rats and Mice. Observations of rats versus mice show differences in metabolism, both quantitative and qualitative. It has been found that the B6C3F1 mouse metabolized CE to a greater extent and generated even more macromolecular binding than expected when compared to the Osborne-Mendel rat. Also noted in the mouse was a lack of DNA alkylation[↓], an increased hepatotoxicity, and an increase in DNA synthesis. This led to the proposal that CE was probably not initiating liver cancer in the mice through alkylation of DNA, but was increasing tumor formation through a recurrent injury (i.e., a cytotoxic type of mechanism). The importance of this proposed mechanism is that chronic, hepatotoxic doses would be required to induce cancer in any species.

Acute and Chronic Toxicities of CE. The acute and chronic toxicities of CE have been adequately summarized in a number of reviews and the data used here are drawn largely from them. The acute lethal effects (LD_{50}) of CE occur at moderately high concentrations (g/kg):

Oral in Rats	5-7
Oral in Dogs	6
Intraperitoneal Injections in Mice	3
Intraperitoneal Injections in Dogs	2.8

The NOAEL vapor concentrations after 7 hr/day, for 5 days/week, for 6 months are:

- 100 ppm (0.5 g/m³) in guinea pigs.
- 200 ppm (1 g/m³) in rats and rabbits.
- 400 ppm (2 g/m³) in monkeys.

Likewise, 30 exposures each of 8-hr duration to 700 ppm (3.5 g/m³) or continuous exposure to 200 mg/m³ for 90 days produced no visible signs of toxicity in rats, dogs, monkeys, guinea pigs, or rabbits.

Like other chlorinated aliphatics, when administered at acutely toxic levels CE is principally a central nervous system depressant. It also produces some liver and kidney damage, but at doses far higher than those at which carbon tetrachloride produces similar damage. In rodents, liver injury occurs after a single dose at about 2 g/kg. CE, like many chlorinated solvents, may sensitize the heart to epinephrine such that stress or excitement may produce cardiac arrhythmias.

Teratogenicity and Reproductive Toxicity. CE produced no teratogenic effects in either rats or mice exposed for 7 hr/day at 300 ppm (1.5 g/m³) on days 6-15 of

gestation. In this study the indices monitored for adverse effects were the number of implantations, litter size, incidence of resorptions, fetal sex ratios, fetal body measurements, and morphologic anomalies. It was concluded that CE was not significantly maternally toxic, embryotoxic, fetotoxic, nor teratogenic in this study. Another study has reported that male mice exposed to 2000 ppm (0.2 percent in air) for 4 hr/day for 5 days had 2.4 percent abnormal spermatozoa compared to a 1.4 percent abnormal sperm count in controls. While this was a statistically significant difference, no change was observed at the 200 ppm exposure and the relevance of the 2000 ppm exposure in mice to expected human exposures, if any, is minimal.

Mutagenicity. CE has been reported as weakly positive or positive in only a few mutagenicity tests, but negative in a number of others. The role that might have been played by contaminants in some of the positive tests has been suggested, but not proved. Therefore, evidence of the mutagenic potential of CE is considered to be inconclusive and confounded by the presence of mutagenic contaminants in a number of studies using the technical grade compound. Pure CE is apparently not mutagenic.

Carcinogenicity. The National Cancer Institute published results of its bioassay in 1978. B6C3F1 mice had been administered CE 5 days/week for 78 weeks. The results are in the table below.

Hepatocellular Carcinoma Rates among Mice, according to Dose			
Male		Female	
1.2 g/kg day	2.4 g/kg day	0.9 g/kg day	1.8 g/kg day
26/50	31/48	4/50	11/49
In control animals the rate was 1/40 when both sexes were combined.			

In Osborne-Mendel rats exposed to doses of either 0.6 or 1.2 g/kg day, no liver cell tumors occurred and there was no statistically significant increase for any other type of tumor. The rat data are complicated somewhat by the poor survival of all groups during the course of this particular bioassay. Likewise, the positive result in the B6C3F1 mouse is somewhat undermined by the fact that about 0.3 percent of the CE consisted of the mutagenic contaminants common to technical grade preparations. Subsequent studies in these strains of rodents yielded essentially the same results.

An inhalation study performed by an independent laboratory reported that CE again produced positive results in the B6C3F1 mouse but was not carcinogenic in the Osborne-Mendel rat. A third study using rats also yielded negative results. Dermal and oral administration of CE to ICR/Ha Swiss mice has also yielded negative results. Finally, no increased incidence of tumors was found with an 18-month inhalation exposure to 100 ppm (500 mg/m³) or 500 ppm (2.5 g/m³) of nonepoxyde-stabilized CE in hamsters, rats, and mice. Thus, even when using technical grade CE with mutagenic contaminants present, CE was found to be positive in only one strain of mice.

[↓] A radical with the general formula C_nH_{2n+1} such as methyl, ethyl...

2.7.9.3. Summary of the Toxicology of CE in Humans

The acute toxicity of CE in humans is remarkably similar to that observed in animals and will not be discussed here for the sake of brevity.

Chronic Toxicity Produced in Humans. Of two epidemiologic studies examining the cancer mortality in occupationally exposed persons, one study reported a lower-than-expected total mortality (49 versus 62) and cancer mortality (11 versus 14.5) for 600 male workers exposed to 50 ppm of CE. The other study reported a cohort of 2117 CE-exposed workers. Again, both total mortality and cancer mortality were lower than expected based on rates established for the general population. Out of these two studies some 1050 workers could be identified who had been exposed to CE for at least 15 years at an exposure level of about 50 ppm or greater.

2.7.9.4. Extrapolation of the Animal and Human Data

Both threshold and non-threshold models might be considered for the animal data. Since a significant human database is available, risk estimates can be generated from these data as well, and then used in comparison with the animal extrapolations and as a guideline for final recommendations.

For non-cancer toxicity, or threshold situations, models for extrapolating to no risk are reasonably straightforward and basically are similar to the suggested no-adverse-response level (SNARL) developed by the National Research Council of the National Academy of Sciences or the no-observable-effect limit (NOEL) of the EPA. Calculations are applied that make the conversion from a no-effect level in animals to a **safe upper limit of exposure to humans** (i.e., the human threshold dose). The conversion must also take into consideration such factors influencing species response as absorption, metabolism, and excretion. Finally, a **safety** or **uncertainty factor** is applied, which is derived from an evaluation of the extent, reliability, reproducibility, and interspecies variability of the available data from animal and, when available, human studies.

2.7.9.5. Safe Human Dose Threshold Model using Non-cancer Animal Data

A review of the animal data on CE toxicity listed earlier, shows that the *best estimate* for an animal-derived no-observable-effect level (TD_0) is 35 ppm. This can then be used as a threshold dose in a risk assessment formula for human exposure to CE. The particular formula we will use here is

$$ADI = \frac{ThD \times BW \times AF \times ER \times t\%}{SF}$$

ADI = acceptable daily intake;

ThD = the threshold dose for which no observable effect was produced in the animal species;

BW = body weight of human (average assumed to be 70 kg);

AF = absorption factor, the percentage of the dose absorbed via the designated route of exposure in the animal species divided by the percentage of the dose absorbed by humans via the designated expected route;

ER = exposure ratio, the dosage regimen (i.e., rate of exposure) in the animal species divided by the anticipated exposure in humans;

$t\%$ = a ratio of the elimination half-life in the animal species to the elimination half-life for humans;

SF = a safety factor included to dampen individual variability in responses to chemicals; SF depends upon the reliability of the database used for the extrapolation. A value of 10, 100, or 1000 is selected as shown below:

10 = When the extrapolation is derived from a good database of human origin and there exists a substantial animal data base.

100 = Human data are scanty, inconclusive, or absent; long-term, reliable animal data are available. No long-term human data are available; animal data are scanty, or interspecies sensitivity varies greatly.

The BW factor is given a value of 1.0 rather than 70 because it is assumed that for an inhalation exposure at steady state the concentrations in critical tissues will be similar for all species and there will be no size adjustment needed.

The AF is assumed to be 0.70 even though this value is probably similar for all species and would therefore be 1.0 when making animal-to-man comparisons. It is included as 0.70 in order to provide an additional margin of safety in this calculation.

The ER is 1.0 in as much as exposure situations are set to be the same in animals and humans.

The ratio $t\%$ is assumed to be 1.0.

The SF is set at 100 and is derived from a factor of 10 because good human data exist that suggest a ThD of 35 ppm does not lead to chronic illness in humans, but since chronic animal data were not utilized, and as the actual ratio for $t\%$ cannot be calculated because the *human half-life is not known*, a second factor of 10 is included to insure safety for a heterogeneous human population.

Thus, substituting in the equation:

$$ADI_{air \text{ continuous exposure}} = \frac{35 \text{ ppm} \times 1.0 \times 0.7 \times 1.0 \times 1.0}{100}$$

$$\text{or, } ADI = 0.245 \text{ ppm in air, or } 1.225 \text{ mg/m}^3.$$

To convert this value to a safe **water concentration** it is assumed that a 70-kg man will inhale about 24 m^3 of air per day and consume 2 liters of water per day. Thus the ADI becomes

$$ADI_{water} = \frac{ADI_{air} \times 24 \text{ m}^3}{2 \text{ l/day}} = \frac{(1.225 \text{ mg/m}^3)(24 \text{ m}^3)}{2 \text{ l/day}} = 14.7 \text{ mg/l} = 14.7 \text{ ppm.}$$

This means a daily intake of ~ 30 mg/day.

2.7.9.6. Safe Human Dose Threshold Model using Non-cancer Human Data

This second approach uses human data. An Estimated Permissible Concentration (EPC) can be calculated by using safe occupational air concentrations as a guideline. Essentially, the EPC for air is calculated by converting the amount of CE a worker theoretically can be exposed to safely during a 40-hr week to the same total amount of CE but derived from a continuous daily air exposure (i.e., 40 hr/168 hr per week). A safety factor of 100 is recommended in calculating an EPC. The EPC for water is then derived by applying the same conversion as was made for the ADI calculation in the preceding section.

The occupational threshold limit value (TLV) for CE is 100 ppm (500 mg/m³) for an 8-hr shift; thus the EPC for air is calculated to be:

$$\text{EPC}_{\text{air}} = \frac{(500 \text{ mg/m}^3)(40/168)}{100} = 1.19 \text{ mg/m}^3.$$

Using the same extrapolation as before to convert safe air exposures to safe drinking-water exposures we get

$$\text{EPC}_{\text{water}} = \frac{(1.19 \text{ mg/m}^3)(24 \text{ m}^3)}{2 \text{ l/day}} = 14.3 \text{ mg/l} = 14.3 \text{ ppm.}$$

Note that the techniques used to extrapolate risk from the animal and human data produce very similar values.

2.7.9.7. Safe Human Dose Non-Threshold Model using Cancer Animal Data

For the nonthreshold (i.e., cancer) risk estimates, several different models can be chosen and each will derive a slightly different number. The linear interpolation model proposed by Gaylor is selected here because it probably represents the most conservative approach and essentially derives the upper bound of the risk estimates that would be arrived at in other ways in other models. The formula for deriving the risk estimate is

$$\text{SHD} = \frac{Rd_e}{UCL}$$

where

R = the conventional *acceptable* probability that cancer will occur;

d_e = the experimental dose chosen as reference in the animal studies;

UCL = the upper confidence limit cancer incidence observed at the reference;

SHD = the safe human dose.

If the acceptable risk (R) is set at 10⁻⁵ or 1/100,000, and if we use the *lowest positive dose* for male mice from the NCI cancer bioassay data and the upper bound of the 95 percent confidence interval (0.50), the safe human dose can be calculated as

$$\text{SHD} = \frac{(10^{-5})(1200 \text{ mg/kg day})}{0.50} = 0.024 \text{ mg/kg day.}$$

Extrapolating to a 70-kg man, and assuming a human lifetime exposure of 70 years versus 2 years for a rat, the safe human daily intake (ADI) becomes:

$$\text{ADI} = \frac{(24 \mu\text{g/kg day})(70 \text{ kg})}{35} = 48 \mu\text{g/day.}$$

If humans are assumed to consume 2 liters of water per day, then the acceptable water concentration, based on animal data for the extrapolation purposes, is:

$$\text{Water Concentration Limit (2 l/day)} = 24 \mu\text{g/l or } 24 \text{ ppb.}$$

2.7.9.8. Safe Human Dose Non-Threshold Model using Cancer Human Data

In the case of CE, the human data do not show an observable increase in the cancer rate. But using "0" in calculations is not practical. Statistically, the 99 percent upper confidence interval for a test population of 1000 individuals is 0.45 percent. That is, the actual cancer rate could be as high as 0.45 percent (0.0045) or 4.5/1000 persons exposed and, statistically, once in a hundred times, it will be mistakenly identified as zero.

Assuming this worst case when no cancer risk is detected in 1000 exposed individuals, the actual undetected risk could be as high as 4.5 × 10⁻³.

There exist two epidemiologic studies based on occupational exposures for CE. In both, the total mortality and cancer mortality were less than expected, so the risk observed can be considered to be zero. If we combine the populations from both studies, which had approximately the same CE exposures, we may make the following assumptions and calculations:

- There were 1050 persons exposed to about 50 ppm in an occupational setting.
- The average daily exposure was 1.4 g/day (about 2.0 g/day for a 5-day work-week; conversion to a 7-day week will need to be made for daily environmental exposure).
- The 99 percent upper confidence limit on the risk for this population is approximately 4.5/1000 or 4.5 × 10⁻³.
- It was assumed that the average exposure interval was only 15 years; thus, this estimate will need to be converted to a lifetime of 70-yrs.

- The ADI is contained in 2 liters of water, which is the amount of liquid a person consumes each day.

Since the risk for the 1050 persons exposed to 50 ppm of CE is equivalent to $4.5 \times 10^{-3} = 1.428 \text{ g/day}$, a 10^{-3} risk will be

$$\frac{1428}{4.5} = 317 \text{ mg/day}$$

Since the 10^{-3} risk = 317 mg/day after only 15 years of exposure to CE, a 10^{-3} risk for a lifetime exposure (70 yr), given the same dose, would be

$$317 \text{ mg/day} \times (15/70) = 70 \text{ mg/day};$$

and a 10^{-5} risk, with daily exposure for a lifetime, would be **700 µg/day**. Therefore, assuming 2 l are consumed per day, the safe water concentration for a 10^{-5} risk estimate is 350 µg/l (or ppb) if human epidemiologic data are used for extrapolation purposes.

Note that our hypothetical risk has been calculated from a substantial body of negative data by making worst-case assumptions that reflect the actual data. A hypothetical risk has therefore been calculated to help set an exposure guideline even though no such risk has been identified in humans.

2.7.9.9. Recommended Groundwater Standard

Possible water Standards, using threshold model:

	Safety factor of 100	Safety factor of 1000
1. Threshold derived from animal data	14.7 mg/l	1.47 mg/l
2. Threshold derived from human data	14.3 mg/l	1.43 mg/l

The values are very similar; the similarity stems from coincidental use of nearly equivalent inhalation levels obtained from animal experiments and time-established human occupational guidelines. These rather high values for the CE content of water reflect the fact that CE is not a potent toxicant at acute exposures. The few studies that reflect its noncancerous chronic toxicity likewise suggest that it is a relatively unremarkable toxicant at other than high concentrations. As was seen earlier, while there is animal evidence demonstrating that CE increases the tumor incidence in the B6C3F1 mouse, these studies are mitigated by the fact that CE is not carcinogenic in the rat, or hamster, or in another strain of mice. The positive studies are also confounded by the fact that contaminants in technical-grade CE are mutagenic and may have contributed significantly to the response observed in the susceptible mouse.

Studies comparing metabolic and biochemical differences between susceptible and nonsusceptible species for CE-induced oncogenicity suggest that the susceptible strain of mouse is not a relevant animal model for predicting human risks. In

addition, a ranking of CE using the system proposed by R. A. Squire indicates that CE would score approximately 31 points, which is equivalent to the score for saccharin. This score suggests that CE is not a likely human carcinogen, especially at low doses. Epidemiologic evidence, while perhaps not yet of sufficient strength to eliminate all concern for the carcinogenic potential of CE, indicates that even for the worst possible case it is a relatively weak human carcinogen. Thus, there is much evidence to suggest the values in the preceding table are adequate for the protection of health in the circumstances of chronic ingestion of CE and would adequately protect human health from all adverse effects. However, cancer data were also considered so as to calculate levels in the event that additional evidence better defining the carcinogenicity of CE is produced.

The values for the estimated safe water concentration, based on carcinogenic potential, are listed below.

Possible water Standards, using non-threshold model:

	10^{-5} Risk	10^{-6} Risk	10^{-7} Risk
1. Estimates derived from animal data	24.0 µg/l	2.40 µg/l	0.24 µg/l
2. Estimates derived from human data	350 µg/l	35 µg/l	3.5 µg/l

In both of the tables of extrapolated standards, the risk estimates generated from the animal data can be considered reasonably conservative estimates. Gaylor's model has been applied to the second table, which is based on the assumption that CE is carcinogenic; the animal data in that table were considered to give a worst-case estimate of the carcinogenic potential of CE. That estimate was then tested for its relevance to the human situation by extrapolating to, and identifying for comparison, the exposures that would be predicted to produce measurable cancer rates in humans if the base estimates of risk were correct. This test of the data is an upward linear extrapolation from the dose representing the 10^{-5} risk estimate. When this is done, the additional increase in cancer for persons occupationally exposed should be about 17 percent for an exposure, over a 15-yr work interval, to 50 ppm of CE. This incidence is very high and would have been observed in the two actual epidemiologic studies that were made of workers with similar exposure levels and exposure intervals. It can be concluded then that the risk estimates generated here from the animal data do overestimate the human risk. A similar test of the extrapolation made from the human data in the two actual epidemiologic studies cannot, of course, be done, but the true risk of CE exposure can be no worse than the estimate derived from the human data in which a cancer incidence was assumed for negative data. Therefore, if it is assumed on the basis of the animal data that CE might carry some carcinogenic potential and risk, a final standard for groundwater could be reasonably chosen as that value corresponding to a previously determined "acceptable risk" level (i.e., 10^{-5} , 10^{-6} , or 10^{-7}) using the extrapolation of the human epidemiologic data.

2.7.9.10. Summary of the Estimates of Risk

It is recognized that risk management is a process in which environmental standards should reflect a variety of important considerations when they are established for contaminant levels in potential human exposure sources such as water. Of primary importance is the protection of human health. Once this criterion is satisfied a regulatory standard may reflect other societal concerns as well. For example, while it could be argued that 1 mg/l might adequately protect human health from CE exposure, if subsequent human or animal studies removed concerns for its carcinogenic potential, the value chosen for a standard might be lower and reflect society's desire for a chemical-free drinking water source. For comparative purposes the EPA has established federal primary drinking water standards for a number of chlorinated organic solvents and chlorinated pesticides. *These standards range from the 1-ppb to the 100-ppb level.* Since the 10⁻⁵-risk estimates calculated from the animal and human data for our example are reasonably conservative, and generally bracket this range, the state might argue to set the standard somewhere between 100 and 350 ppb. Such a standard should reasonably and safely protect human health within the limits of the uncertainty that are inherent in the database and the models used to calculate the values from it. It would also be in keeping with the intent of the EPA and the guidelines proposed by that agency for other chlorinated aliphatics. Reiterating that much data are available to greatly lessen concern for the human cancer risk posed by CE, a state Division of Environmental Resources might reasonably select the cancer-risk estimate for a drinking-water standard as high as 350 ppb and at the same time choose to adopt the threshold-derived values in the 1-2-ppm range as a reasonably safe guideline to protect aquatic life and human health under short-term unexpected situations such as spills into rivers acting as drinking-water supplies.

It is hoped that the reader will appreciate the fact that should the state finally pick either level as a standard, various interest groups could argue against one or the other of them by reasoning either that

- (1) risk of carcinogenic potential must be assumed until better and more definitive evidence proves there is none, or
- (2) since the animal and human data indicate no cancer risk, the higher value is safe because apparently-safe occupational exposures are far higher.

It may interest the reader that currently OSHA allows up to a **500 mg/day** exposure to CE-type compounds in the workplace, while the EPA, which has not proposed a formal standard, has recommended about a **60 µg/day** exposure. These are clearly very different exposures.

This difference is caused because the EPA criteria are based upon the extrapolation of animal carcinogenicity data, and typically the EPA does not consider modifying factors such as mechanistic and species variations in the carcinogenic response; nor does it use human data as a test or modifier of the animal extrapolation.

Thus, while the OSHA standard is designed to protect workers occupationally exposed for perhaps most of their lifetime, the EPA, by virtue of its risk assessment methodologies, advocates an exposure that is some **60-70,000 times lower**.

Such a disparity between standards or recommendations for federal regulatory agencies is common today, *because each agency assesses and manages risk by a different process.*

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Targets - Bio-transformation

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3. Targets • Bio-transformation

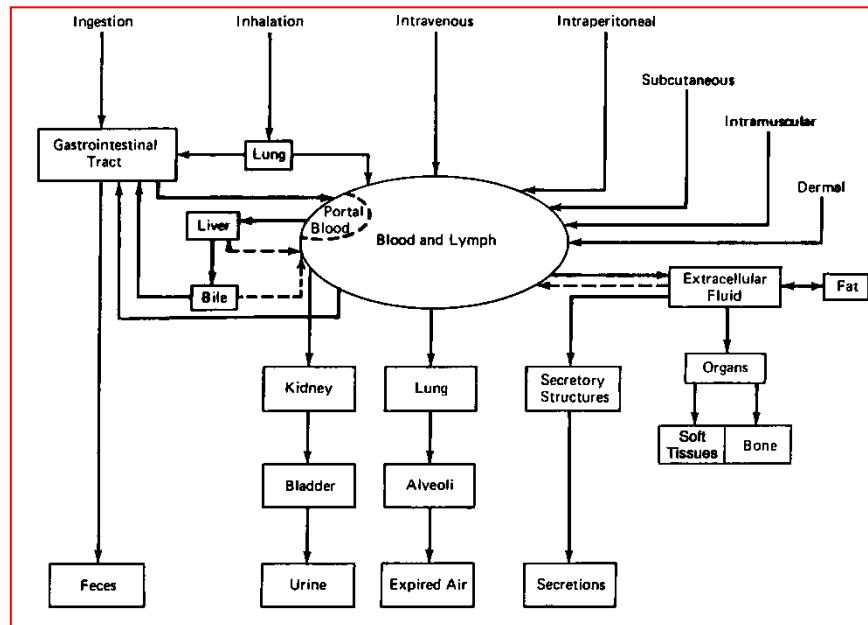
3.1. Routes of Absorption

Toxicants access the body through many absorption routes, spilling into lymphatic and vascular compartments (F3.1). The intravenous route allows complete and immediate penetration, whereas other routes allow delayed and incomplete absorption. The general hierarchy, ordered in decreasing absorption, is:

Injection > Inhalation > Contact on mucosa > Ingestion

External barriers, such as the skin and the gastrointestinal tract, are quite complex. Internal barriers, as the blood-brain and lung-blood barriers, may have a thickness of only 1 cell, as little as 1 μm . Different routes of exposure (inhalation, cutaneous, ingestion) may result in different amounts of toxicants circulating in the bloodstream, as well as various levels of toxicity. This relates to the toxicant's physical and chemical properties, anatomy (thickness of the membranes, size of internal reservoirs) and biochemistry.

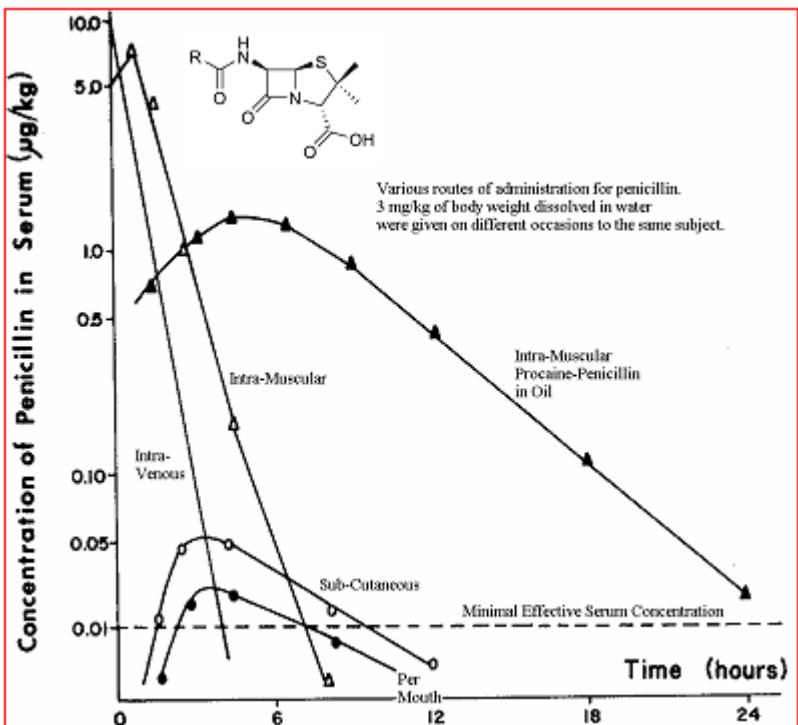
Different routes of administration of drugs result in notably different blood levels over time, as seen in F3.2. In this graph, determining influences are the active excretion of penicillin by the organic acid system of the kidneys, and penicillin's binding to albumin. In some of the curves in the graph, the level in the blood is maintained by oil-phase, sub-cutaneous or enteric compartments.



F3.1. General paths of toxicants through the body.

Casarett & Doull's.

If one views the body as a multitude of membrane layers through which a toxic agent penetrates to reach a site of action, one can anticipate that the variables governing passive diffusion and active transport control the rate and extent of penetration. There may be a large factor (1,000-10,000) between the concentration of a toxicant circulating in the blood and that found at a target site.

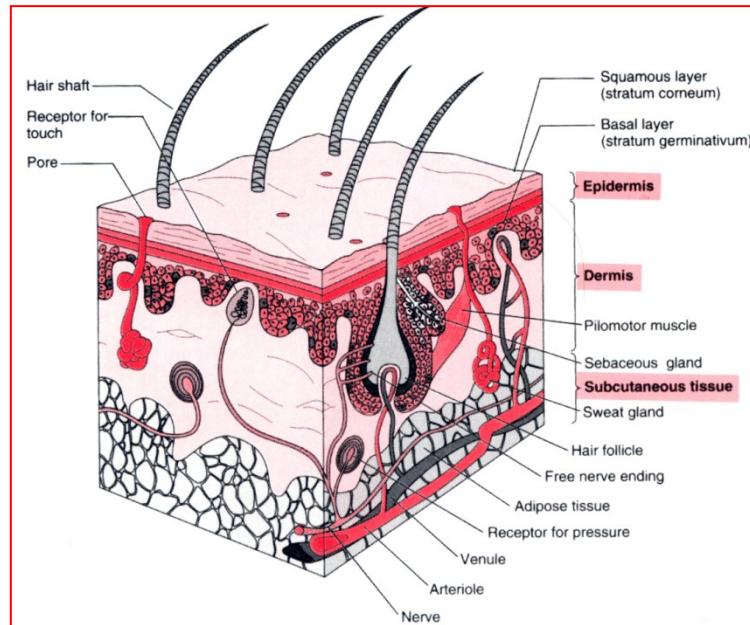


F3.2. Concentration of Penicillin in the blood following various modes of administration. *Pharmacology: Drug Actions and Reactions, 1978.*

3.1.1. Skin

The skin (F3.3) has many roles:

- ✚ Keeps out toxicants, UV and micro-organisms.
- ✚ Biotransformation and detoxification.
- ✚ Elimination of toxicants through sweat.
- ✚ Temperature and fluids regulation.
- ✚ Sensory reception (temperature, pressure, pain).



The *stratum corneum* is only 15-20 dehydrated cells thick. These cells are keratinized¹, extracellular lipids forming a barrier. The thickness of these barrier cells varies tremendously (anticubital fossa vs heel).

Skin penetration is usually through passive diffusion. For example, when swimming or taking a bath or shower, absorption is substantial. Skin is vulnerable to damage, and its impermeable properties can easily be lost. Toxicants that are small, non-polar and lipid-soluble will diffuse most rapidly. Skin is vulnerable to organic solvents such as chloroform, methanol and dimethyl sulfoxide because of dissolution of skin lipids.

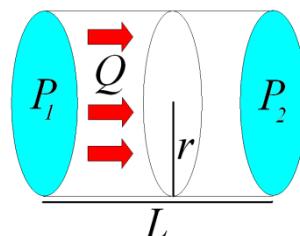
¹ In mammals there are soft epithelial keratins (cytokeratins), and harder hair keratins. As certain skin cells differentiate and become cornified, pre-keratin polypeptides are incorporated into intermediate filaments. Eventually the nucleus and cytoplasmic organelles disappear, metabolism ceases and cells undergo apoptosis as they become fully keratinized.

3.1.2. Lungs

Lungs (F3.4) are a very deep, humid cavity that is difficult to clean. Mucus-secreting cells and a ciliated epithelium cooperate to achieve a particle removal half-life of 30-300 min. Coughing can shorten the transit. The movement of the cilia in the lungs is coordinated. One cell may have as many as 200 cilia. The “muco-ciliary escalator” keeps alveolar tissues clean of particles and of many chemicals that absorb readily into the mucus. If clearance of the mucus from the peri-alveolar region is impaired for some reason, the flow of air is impaired into and out of the lungs.

There is a critical physical limit to the flow of air in and out of the lungs (Poiseuille's Law):

$$Q = \frac{\pi \Delta P r^4}{8 \eta L}$$

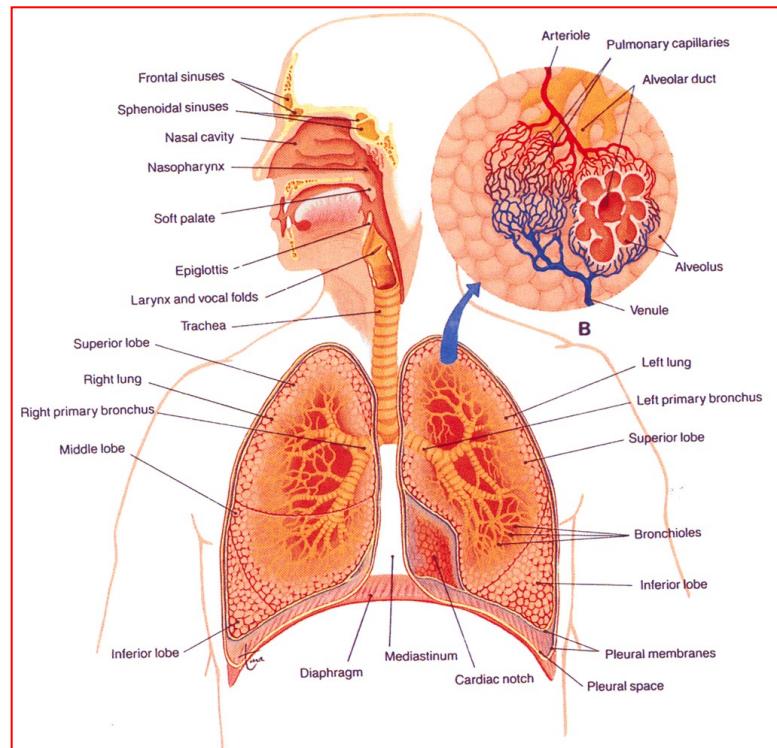


Q is air flow,
 ΔP is pressure difference across the length of bronchiole,
L is length of the bronchiole,
 η is viscosity (air 190 μ -poise, water is 10,000 μ -poise),
r is bronchiole radius.

Notice that if bronchiole radius (r) is reduced by a factor of 2, the air flow is reduced by a factor of 16, for a given pressure difference. This explains the importance of micro-airway diameter in asthma attacks.

- ✚ Normal respiration volume is about 0.5 liters at 12 respirations/minute = 6 liters/min.
- ✚ You can live for a short time on 1.5 liters/minute.
- ✚ The spontaneous rate of respiration is controlled by

centers detecting pH and CO₂ concentration at the base of the brain. Oxygen concentration is less important.



F3.4. General anatomy of the respiratory system.

Essentials of Environmental Toxicology, Taylor and Francis, 1996.

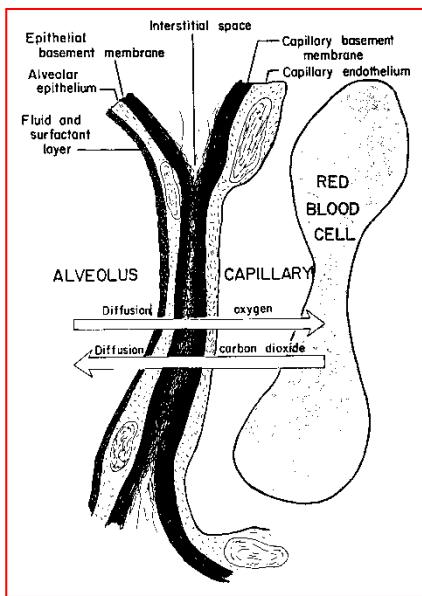
- ✚ The rate goes up to 50 liters/min in high level exercise. So, when working strenuously, transfer of toxicants by this route may be considerably enhanced.

There are about one billion alveoli in the lungs, equivalent to a 70 m² effective surface, which allow the exchange of gases, primarily oxygen and carbon dioxide. Note that water soluble gases such as HCl or formaldehyde normally do not make it

into the alveoli, as they are dissolved into the mucus while going down the bronchial tree.

Only particles less than 1 μm make it down into alveoli, larger

particles being retained at higher levels in the respiratory tract.



F3.5. Epithelial basement membrane of the lung

The distance to be covered between alveolus and capillary is only about 0.5 μm . The alveolar epithelial cell is also called a *pneumocyte*.

Q: What happens to a particle of asbestos lodged in the alveolus?

A: White blood cell either

from the blood or already in the surface of the alveolus will attempt to phagocytize the particle, and enzymatically digest it.

Blood-soluble toxicants entering the lung will be transported along with oxygen to the heart through the pulmonary vein. The heart then distributes the toxicant to all parts of the body.

3.1.3. Digestive System

The process of digestion breaks up food mechanically and chemically into useable elements in great part by hydrolysis, which is why you need extra water after eating.

F3.6. Digestive system.

Essentials of Environmental Toxicology, Taylor and Francis, 1996.

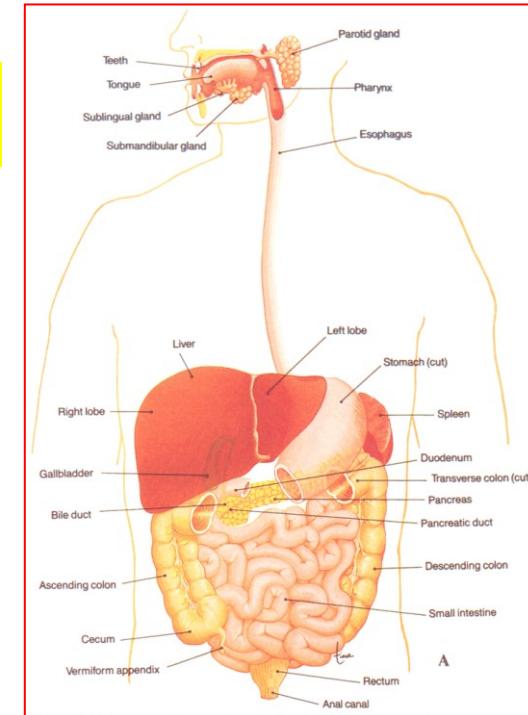
The digestive surface is wet and functions well both in absorption and secretion.

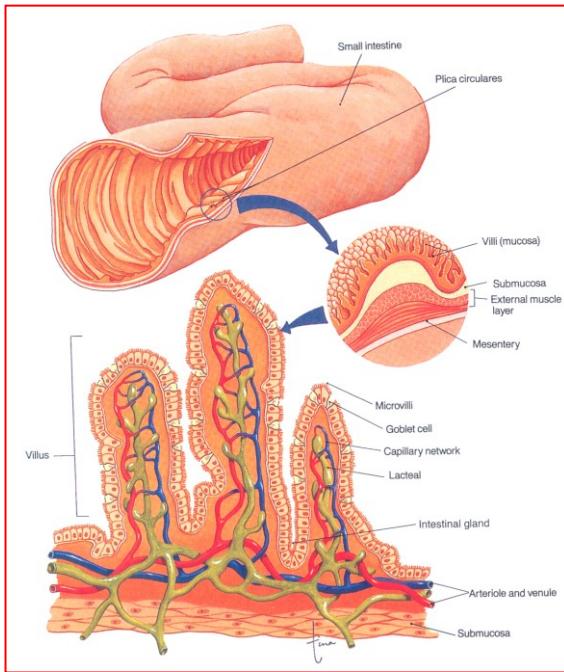
Low pH from HCl secretion is observed in the stomach (pH ~2), where bacteria are killed and protein denatured to make them more sensitive to pepsin degradation.

The pancreas has both an exocrine (pancreatic enzymes for digestion) and an endocrine (insulin control over absorption of glucose into cells) function.

Both bile and pancreatic juices are injected into the duodenum, and they need a slightly basic medium (~ pH 8) for optimal function.

Surprisingly, you can live with only a small length of intestine. Although the small intestine usually has few bacterial colonies, the colon is rich with them. This explains why very small amounts of antibiotics can increase the growth rate of many farm animals (and foster antibiotic resistance), as they make nutrients more available to the animal, as opposed to the bacteria.





F3.7 Anatomy of the small intestine.
Essentials of Environmental Toxicology, Taylor and Francis, 1996.

The inner geometry of the small intestine is such that the epithelium is at mechanical risk. It is routinely sloughed off (~every 5 days in the colon), so that regeneration must be continuous. Most absorption of food and toxicants

is in the small intestine. A notable exception is alcohol, a small molecule absorbed directly in the stomach. In F3.7, the central duct (green) connects with the lymphatic system, ultimately meeting the blood near the aorta. But a toxin absorbed through the intestinal vein goes to the portal vein and directly into the liver.

3.2. Target Organs

3.2.1. Perfusion of organs

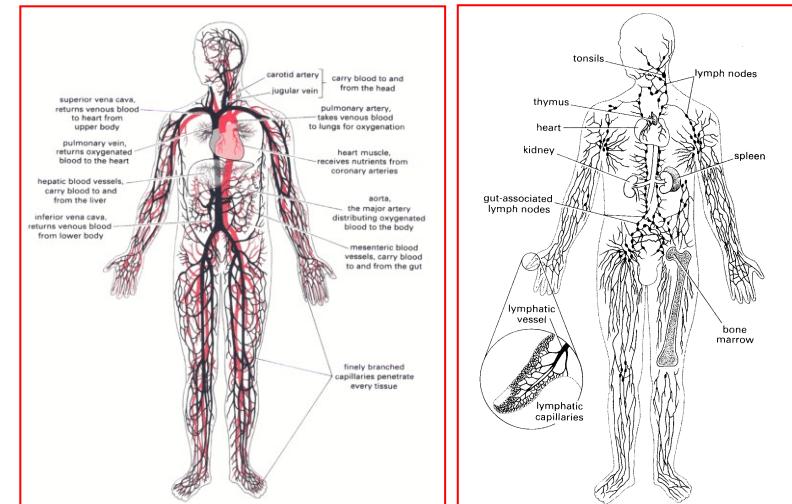
Toxicants act locally, but are also carried by body fluids. The major fluids of the body, the blood and the lymphatic fluid, (F3.9) have a big influence on where toxicants will act. Very avascular tissues, like the lens of the eye, are less likely to be exposed to fluid-borne toxicants than other tissues. This is because the toxicant is likely to be disposed of before reaching

them, or, at least, any crests of exposure are highly attenuated, which implies reduced toxicity.

Region	Mass (kg)	Blood flow		Total cardiac output (% of total)
		(mL/min)	(mL/100g/min)	
Liver	2.6	1500	58.0	27.8
Kidneys	0.3	1260	420.0	23.3
Skeletal muscle	31.0	840	2.7	15.6
Brain	1.4	750	54.0	13.9
Skin	3.6	462	12.8	8.6
Heart muscle	0.3	250	84.0	4.7
Other body	23.8	336	1.4	6.2
Total body	63.0	5400	8.6	100.0 ^a

F3.8. Blood perfusion of various body regions.

Medical Physiology, 1961.



F3.9. Blood and lymphatic circulations.

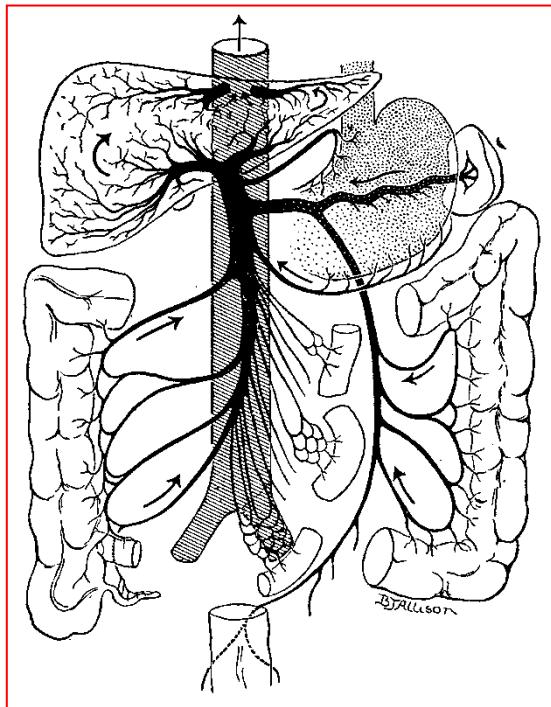
Essentials of Environmental Toxicology, Taylor and Francis, 1996.

3.2.2. First Pass Effect

For any drug or toxicant reaching the blood, we should be concerned with what is known as the “first pass effect”. Because 28 % of the blood circulates through the liver in dense capillary beds at low pressure and flow, and because the liver

has such good extractive capabilities, the agent may be metabolized as it passes through the liver for the first time (F3.10). Approximately 70 % of the blood supply to the liver comes from the portal vein and 30 % via the hepatic artery. In many cases, if the level of the agent in the blood (acquired by ingestion, inhalation or dermal exposure) is "low", little or none will escape into the systemic blood (vena cava) on the other side of the liver. It will be retained and metabolized locally, even up to "moderate" concentrations of the agent, because of the liver's high filtration capacity. This may result in *hepatotoxicity*.

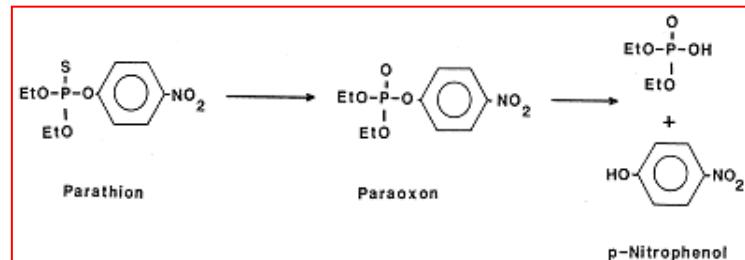
At high levels of exposure and elevated concentrations in the bloodstream, some agent will escape entrapment in the liver and will be forwarded through the *vena cava* to the heart and the systemic circulation. Toxicity may result from the "escaped" fraction reaching other organs.



F.3.10. Incoming arrows show circulation from the digestive system into the portal vein, while the vertical arrow at top shows exit to the vena cava.

As an example, the insecticide parathion must undergo oxidative desulfuration (using the non-specific mono-oxygenase aryl hydrocarbon hydroxylase, or AHH, also known as CYP1A1) to the much more reactive

anticholinesterase (acetylcholinesterase-inhibiting) agent, paraoxon, before it can be degraded further (F3.11). At low-to-moderate blood levels, no parathion will escape the liver, all being destroyed *in situ*.



F3.11. Degradation of parathion. Et = Ethane

However, following excessive exposure (suicide attempts, sprayers, field workers, etc.), not all of the parathion is extracted by the liver, some escapes into the general circulation to be biotransformed in tissues. In nerves (parathion is highly lipid soluble), the intermediate, paraoxon, can inhibit acetylcholinesterase, resulting in the accumulation of the neurotransmitter acetylcholine.

Muscarinic, nicotinic and central nervous system toxic signs and symptoms appear (T1.17). It is thought that the systemic toxicity of parathion in poisoning cases arises from the extra-hepatic biotransformation of the parathion not destroyed by the liver.

3.3. Penetration of Barriers

Indiscriminate absorption of foodstuffs and toxicants into the body is prevented by *tight junctions* between cells. In a lot of the epithelium (ie, skin, inside of vessels, respiratory and digestive tracts), *tight junctions* prevent passage of molecules

between cells by simple diffusion (there are no gaps between cells).

The *Blood-Brain barrier* is an example of such an anatomical barrier that is particularly effective, making it difficult for physicians to obtain therapeutic doses of some medications in the Central Nervous System.

For example, manganese, even if absorbed into the blood, is limited in its effects on the nervous system by the blood-brain barrier. Unfortunately, particles can dissolve on the nasal olfactory mucous membrane, then proceed to nerve cells that lead to the olfactory bulb in the brain, where it is redistributed to do neurological damage...

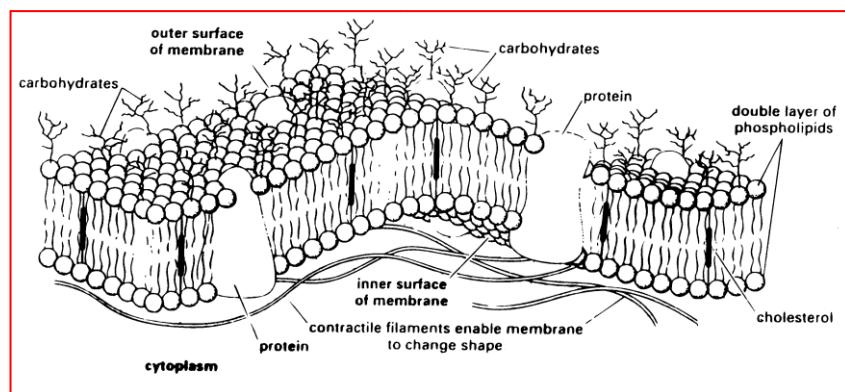
“Pinocytosis” is a mechanism of volume bolus absorption that is active in the gut, but is relatively rare. Down the line, pinocytic particles are expected to meet peroxysomes for molecular digestion. In spite of such marginal absorption mechanisms, however, most toxicants have to enter the body by going through cell membranes...

Proteins have a notoriously difficult time traversing the hydrophobic layers of the plasma membrane. But *Bacillus anthracis* has a clever ways of pushing its proteins through. Anthrax kills with a toxin, a compound composed of three proteins—protective antigen, lethal factor, and edema factor—that somehow penetrate the plasma membrane of the host cell and enter the cytosol, where they make their kill⁵.

Sometimes, a tough cell membrane can be a problem. An organism that infects one-third of the world’s population, *Mycobacterium tuberculosis*, has evolved expert multi-drug resistance primarily by resisting toxicant penetration. The

membrane recipe is a wall of peptidoglycans followed by galactans and arabinans and further by mycolic acids. Most substances cannot diffuse across this boundary. The survival of the bacterium is enhanced by a slow division rate and detoxifying metabolism.

3.3.1. Phospholipid Bilayer



F3.12. PhosphoLipid bi-layer.

TRANSPORT MECHANISMS THROUGH THE BILAYER

1. Passive diffusion (from a concentration gradient across the bilayer): molecular size is paramount only in the case of large molecules (fibers, dusts, peptides, proteins). Since most of the chemicals of concern are of rather small molecular weight, *solubility* is more important. Penetrating substances are lipid-soluble enough to penetrate cell membranes, but not lipid-soluble enough to remain trapped there, such as is the case for the volatile organics that exert an effect on the CNS. The rate of transport (dD/dt) across a membrane is governed by Fick’s Law:

$$\frac{dD}{dt} = \frac{K \times A \times (C_0 - C_i)}{T}$$

A is the cross section exposed to the compound, C_x are the concentrations on either side of the membrane, T is the thickness of the membrane and K is the diffusion constant.

Protein binding to blood and tissue proteins can enhance or retard transport by passive diffusion. Two features, the extent of binding (ie, 30 % or 85 % bound) and the tenacity of binding (loosely or tightly bound) are important. Unbound (free) agents can be transported by passive diffusion, bound agents cannot. To use passive diffusion effectively, a toxicant must be of small molecular size (less than ~600 Dalton), non-polar, and lipid-soluble (Lipinsky Rules).

2. Facilitated transport: mechanisms for sugars (glucose) and amino-acids (vitamins), whose details are not entirely understood. Involves membrane proteins which encourage transfer.

3. Active transport for inorganic ions and for xenobiotics: this process needs ATP. These systems maintain membrane potentials and allows cell-level excretion of xenobiotics (drug resistance).

Most of the chemicals of concern to the toxicologist are relatively small molecules that enter the body and reach critical sites by passive diffusion. A few exceptions include heavy metals (Pb, Cd, Zn, Co), which are acquired by active transport, using carrier systems designed to transport calcium or iron. These transport systems are not sufficiently selective to exclude other divalent cations of roughly the same size and charge (see example, F7.4).

Although passive diffusion always *follows* a concentration gradient, active transport can concentrate agents *against* such a gradient.

- ⊕ The CNS has 2 active transport systems: one for organic acids, the other for organic bases.
- ⊕ The kidney has 2 active transport systems: one primarily for sodium, the other for hydrogen.
- ⊕ The liver has 4 active transport systems: 2 for organic acids, one for organic bases and one for neutral compounds.

The transport processes involved may be carrier proteins (transferrins for iron, etc.) or enzyme systems in which energy is expended. Specificity (sufficient or not) and binding capacity limits are important, as these systems can be saturated. According to the concentration of agent available, up to a certain maximum, the agent will be transported effectively.

Lead, for example, is transported by the same carrier system as calcium and, indeed, competes with calcium. They are both divalent cations and, in calcium deficient states, lead will be absorbed more efficiently. The final "resting place" for lead is in the long bones where one finds much higher levels than are circulating in the bloodstream (up-gradient transport).

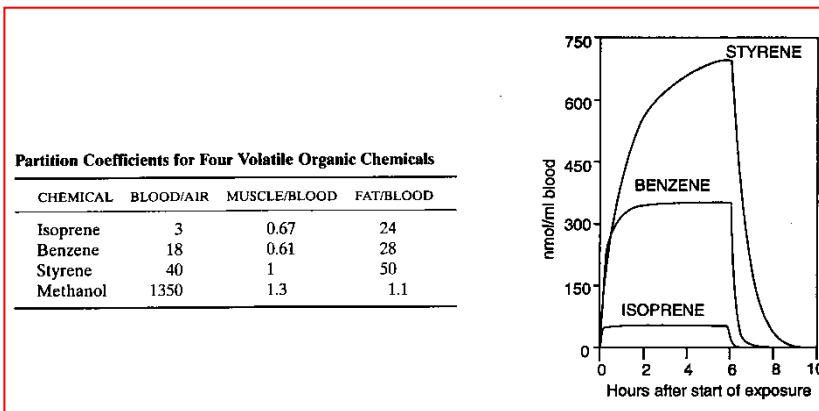
3.3.2. Partition Coefficient

Since membranes must be crossed, the solubility of a drug or toxicant from one medium into the next is a critical determinant of its migration into tissues.

The determination of migrating ability between two media can be specified between any two liquid or solid media, but frequently, reference media are used such as from water to chloroform, hexane or octanol (see below).

$$P_{ow} = \log_{10} \frac{[\text{in Octanol}]}{[\text{in Water}]}$$

In the example of F3.13, one can see that as rats are exposed to breathing various Volatile Organic Compounds, the ones with the larger Partition Coefficient between Blood and Air tend to be drawn out of the inspired air more rapidly and in greater amounts (less isoprene than benzene than styrene in the blood). As well, upon cessation of exposure, some substances tend to remain in the blood for longer periods. However, the decay in a complete animal can also be influenced by fat stores, since the three compounds shown migrate to fatty tissues from the blood (and vice-versa).



F3.13. Effect of blood-air partition coefficient on venous blood concentrations in rats for a 6-h exposure to 2 g/m³ (model).

The partition coefficient (P_{ow}) of a substance between a lipophilic solvent, usually *n-octanol*, and *water* is commonly and conventionally used to quantify substance transfer not only between body compartments but also between compartments in the environment (biota vs air, water or soil).

T3.13a. Hydrophilic - Lipophilic Molecules	P_{ow}
Acetamide	-1.16
Methanol	-0.82
Formic acid	-0.41
Diethyl ether	0.83
p-Dichlorobenzene	3.37
Hexamethylbenzene	4.61
2,2',4,4',5-Pentachlorobiphenyl	6.41

Significant relationships have been demonstrated between P_{ow} and the potential for a chemical to bio-accumulate in the aquatic environment, to concentrate in fish and adsorb on soil and sediments. A low P_{ow} (less than 3) can be used to convince regulatory authorities that an agent is unlikely to bio-accumulate or cause aquatic toxicity.

3.3.3. Dissociation Constant

Since biological membranes tend to give passage preferentially to uncharged molecules, it is important to know the degree of ionization of molecules when dissolved in body fluids. The degree of ionization can be calculated from K_a , the Dissociation Constant of the molecule, and the Henderson-Hasselbalch equation, shown below.

$$K_a = \frac{[H^+] [A^-]}{[HA]}$$

K_a is so named because it refers to dissociation of the acid (H^+) part of the molecule, as opposed to the basic part (OH^-). The Dissociation Constants (K_a) of various substances can be found from tables.

As an application of this equation, let us look at the absorption of acetylsalicylic acid (aspirin) from the human stomach... Remember that $pH = -\log [H^+]$ and $pK_a = -\log [K_a]$.

The pK_a for salicylic acid is 3.

The pH of blood is 7.3.

The pH in a full human stomach could be 4.3.

We use a modified Henderson-Hasselbalch equation:

$$\log \frac{[\text{non-ionized form}]}{[\text{ionized form}]} = \log \frac{[HA]}{[A^-]} = pK_a - pH$$

According to the equation, if $pK_a = pH$, then $pK_a - pH = 0$, and half of molecules are ionized, the other half not; that is, $[\text{non-ionized}] = [\text{ionized}]$.

If we put aspirin in the stomach at pH 4.3, then

$pK_a - pH = 3 - 4.3 = -1.3$, which means that about 5 % of the aspirin is non-ionized ($10^{-1.3}$).

This 5 % of molecules of aspirin is able to cross into the hydrophobic membranes of the stomach wall, and ultimately into the circulation.

When these molecules cross into the intracellular fluid and into the blood, where the pH is 7.3, the ratio of ionized to non-ionized is altered: $pK_a - pH$ is now $3 - 7.3$ or -4.3 , which means that only 0.005 % of the molecules are now non-ionized, and able to cross cell membranes.



This implies that most aspirin molecules that get into the stomach wall (plasma) are unable to diffuse back into the stomach, while there is a continuous 5 % of aspirin molecules able to cross into the stomach wall from the stomach.

Aspirin concentration rises in the stomach wall as a result of that trap, and aspirin is steadily pumped by diffusion into the blood and absorbed effectively.

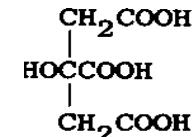
The pH difference between compartments has created a gradient of aspirin concentration.

Since pH is so important to agent penetration, the body has many systems to control it. You can live only for a little while with deviations in pH of about 0.5 units around 7.3.

- pH is stabilized by :
- Buffer systems (bicarbonate, phosphate, protein systems),
- Breathing: CO_2 elimination helps regulate pH,
- Kidneys.

In case you are wondering, the mechanism of aspirin action has been at least partly elucidated: the acetyl group of aspirin seals access to a cavity of the enzyme prostaglandin H₂ synthase ("PGHS"), preventing transformation of arachidonic acid into prostaglandin H₂ (see F3.14).

More complex molecules, such as citric acid (shown), may have many dissociation steps. In the case of citric acid, they occur at pK_{a1} s of 3.14, 4.77 and 6.39.



HENDERSON-HASSELBLACH EQUATION

$$\log \frac{[\text{non-ionized form}]}{[\text{ionized form}]} = \log \frac{[HA]}{[A^-]} = pK_a - pH$$

For Aspirin ($pK_a = 3$):

$$\log \frac{[\text{non-ionized form}]}{[\text{ionized form}]} = \log \frac{[HA]}{[A^-]} = 3 - 7.3 = -4.3$$

Aspirin in Stomach (pH 4.3):

$$\log \frac{[\text{non-ionized form}]}{[\text{ionized form}]} = \log \frac{[HA]}{[A^-]} = 3 - 4.3 = -1.3$$

Aspirin in Pancreatic Secretion (pH 8):

$$\log \frac{[\text{non-ionized form}]}{[\text{ionized form}]} = \log \frac{[HA]}{[A^-]} = 3 - 8 = -5$$

Aspirin in Plasma (pH 7.3):

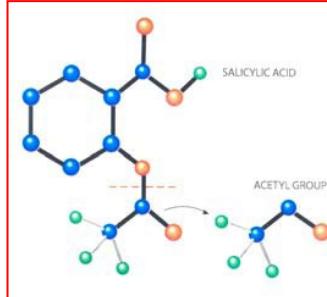
$$\log \frac{[\text{non-ionized form}]}{[\text{ionized form}]} = \log \frac{[HA]}{[A^-]} = 3 - 7.3 = -4.3$$

Computation example (Stomach): $10^{-1.3} = 0.05 = 5\% \text{ Non-Ionized}$

LOCATION	% Non-Ionized	% Ionized
Stomach	5	95
Pancreatic Secretion	0.001	99.999
Plasma	0.005	99.995

Result:

Aspirin is drawn from stomach to plasma and from plasma into pancreatic secretion.

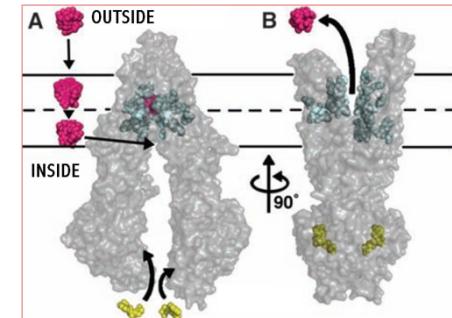


F3.14. Mechanism of action of aspirin.
Aspirin stops an inflammatory cascade which converts arachidonic acid, a fatty acid of cell membranes, into prostaglandins, by inhibition of the enzyme *cyclo-oxygenase*.

At the cellular level, physical and chemical properties may prevent entrance of toxicants inside cells.

If toxicants do penetrate, they can be pumped out by efflux pumps or multi-drug resistance transporters, the same types of pumps responsible for drug resistance in bacteria:

- + Multi-Drug Resistance associated P-glycoprotein (MDR1 gene) is weakly expressed in the liver, but more strongly in the intestines as well as T cells. It is a key factor in the blood-brain barrier, and has been likened to a “hydrophobic molecule vacuum-cleaner”. The diagram at right shows (A) the transport of a red substrate from the membrane to a pocket, and (B) exclusion of the substrate using an ATP(yellow)-dependant conformational change.



- + P-glycoprotein can export hundreds of chemically unrelated toxins, using binding sites in an internal cavity capable of stereoselectivity based on hydrophobic and aromatic interactions¹⁵,
- + the phospholipid export pump (MDR3 gene) translocates phospholipids (from bile, for example) from inner to outer aspect of membranes,

- the conjugate export pump (MRP2 gene) transfers organic anions into bile (conjugates of bilirubin, glutathione, glucuronide and sulfates).

Chewing with metal dental fillings releases mercury and activates these pumps, reducing the exposure of cells. Some chemicals, such as synthetic musks, are capable of specifically disabling these cellular pumps, leaving the cells vulnerable to other exposures⁹.

Once in the bloodstream, toxicants can be taken up by organs specialized to deal with them, or damage organs that are not.

3.4. Distribution

The two Circulatory Systems are by far the most powerful distributors of toxicants throughout the body.

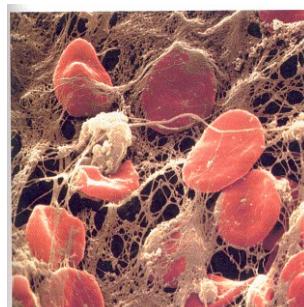
3.4.1. Lymphatic Circulation

The lymphatic fluid can be seen as a yellowish crystalline-like fluid exuding from a cut. It carries away extra-cellular space proteins and particulate matter. It is driven mostly by muscle contraction, but also by a specialized system of valves throughout the system of lymph capillaries (F3.9), using only partly blood circulation as a propellant. Without lymph flow, you could only live for 24-hours.

3.4.2. Blood circulation

F3.15. Red Blood Cells trapped in fibrin clot.

The delivery of toxicants to target organs is primarily from the blood, because of the speed of its transit. Blood is made of *erythrocytes*, *leucocytes*, *platelets* and *plasma*. If fibrin is removed from plasma



(stirring *plasma* with a glass rod will polymerize fibrinogen to it), one gets *serum*.

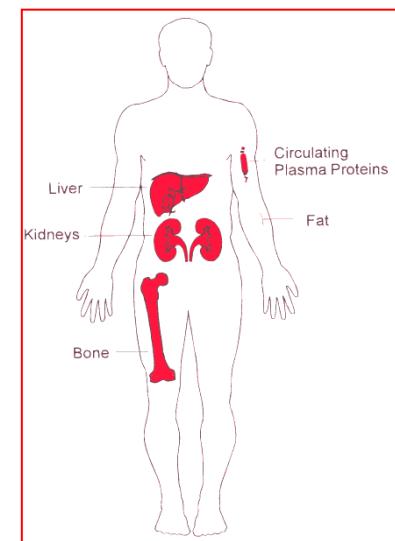
Keep in mind that plasma protein such as **albumin** (normal levels of albumin are 3.5-5 grams/dl) can keep toxicants in the blood for a period of time (binding 0 to 100 %), attenuating the release of toxicants to the organs.

Total plasma volume in the average human adult is **3 liters**, so any soluble toxicant injected into a vein would quickly be diluted into that volume.

Considerations of molecular size and lipid solubility are again of importance in gaining access from the blood into organs. Some chemicals present in the blood find more affinity for specific tissues such as **fat** (DDT) and **bone** (lead), and may concentrate there over time.

F3.16. Typical toxicant storage sites in the body. *Essentials of Environmental Toxicology*, Taylor and Francis, 1996.

Where toxicants end up is of great importance in final determination of toxicity. But controlling drug deposition in the body is difficult. In the pharmaceutical industry, unfavorable “ADMET” (absorption, distribution, metabolism, excretion and toxicity) is responsible for more than half of compound rejections at the level of clinical trials.



3.5. Storage

Storage is a type of *bioaccumulation*. Tissues and animals may bio-accumulate toxic halogenated defenses from compounds

they synthesize (Sea Slug, left) or from external sources such as their diet (*Aplysia*, right).



These chemicals make them unappetizing to predators, including sharks. Some of these compounds have been shown to move up the food chain into the milk of Faroe Island women (from whale blubber), just like PCBs⁸.



Yet other animals get toxins from their prey, and then metabolically upgrade their toxicity (x 5) to protect themselves. This small tropical frog, *Dendrobates*, sports a very toxic skin (*pumiliotoxin*).

3.5.1. Bone

Bone is actually in a state of perpetual regeneration every 7 years. One major constituent of bone is hydroxyapatite $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$. In intoxication, fluoride may substitute for OH (making bones brittle), and radio-active strontium or lead for calcium.

3.5.2. Fat

Body fat is a storage compartment that varies in size, depending on the individual. In dieting, autointoxication can occur as toxic substances are released into the blood.

Volunteers who lost 10 kg saw an increase of 23 % in blood

levels of 14 PCBs and 11 pesticides. These increased concentrations in turn reduced the 15 subjects' metabolic rates either through effects on mitochondria or on the thyroid gland⁷. Since fat is so adept at absorbing a large category of liposoluble compounds, can it be viewed simply as a compartment designed to attenuate the exposures of the remaining body compartments to toxicants?

3.5.3. Resulting Distributions for Lead and Dioxin

As can be seen in T3.17, body tissue distributions may vary substantially between toxicants. Hair and nails have very high concentrations of lead, and are often used to document lead intoxication.

T3.17. Distribution of Pb and Dioxin			
LEAD	%	DIOXIN	%
Bone	95.0	Fat	95.0
Liver	4.4	Liver	1.1
Lung	0.1	Muscle & Skin	2.5
Kidney	0.3	Gut	1.3
Brain	0.2	Other	0.2

3.6. Biotransformation

It has been estimated that 80 % of drugs taken by people emerge from the body intact. But what happens to the remaining 20 % is interesting...

The major function of the various biotransformation pathways in body tissues is to convert relatively lipid-soluble exogenous (and endogenous) agents into water-soluble, nontoxic and readily excreted compounds. The strategy is: make toxicants water-soluble so they will not diffuse back across cell membranes, and will be more easily controllable for excretion.

GENERAL MECHANISM OF BIO-DETOXIFICATION

- + **hydrophilic toxicants** are generally eliminated from the body in their original chemical form,
- + **lipophilic toxicants** must be biotransformed for elimination.

Many of the detoxifications reactions occur within the endoplasmic reticulum of individual cells.

Unfortunately, biotransformation can also result in *bio-activation* or *toxication*, which involves the production of a more powerful toxicant derivative from the original toxicant.

The major organ for detoxification is the **liver**. Detoxification is conventionally segmented into **Phase I** and **Phase II** reactions. These reactions are also used by the body to eliminate normal by-products of metabolism. For example, hemoglobin is metabolized to bilirubin, which goes through the biliary system into the feces. Molecular weights smaller than 250 Dalton are excreted in urine, molecular weights larger than 350 Dalton are excreted in bile and feces.

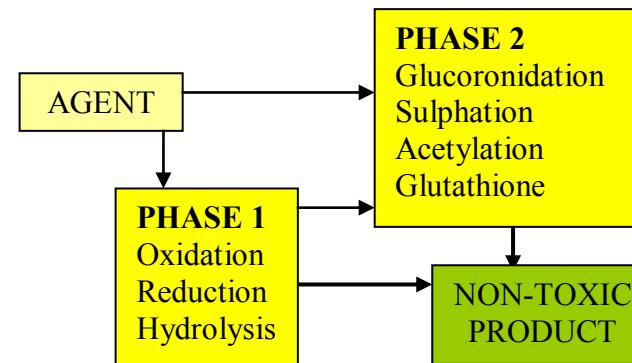
Not all chemicals are processed through both Phases (F3.18). Generally, Phase I puts a molecular *handle* on molecules, while Phase II makes them more hydrophilic.

Heroin and codeine metabolism involve Phase I, acetaminophen (Tylenol) and morphine go directly to Phase II.¹

3.6.1. Variations in Biotransformation

The importance of bio-transformation in biological organisms is underlined by the fact that most proteins are actually

enzymes^v (as opposed to structural, messenger or transport proteins), agents that facilitate the transformation of one chemical into another.



F3.18. Biotransformation pathways.

When a single chemical agent is biotransformed, it is common that multiple forms of the agent result from the biotransformation process. As these reactions are often dependant on enzyme kinetics, exposure concentration may influence the variety of molecular species produced. These multiple forms of the original agent make toxicity more complex.

However, most of the differences in biotransformation are **quantitative** (as opposed to qualitative), resulting from changes in the rates at which toxicants are converted to intermediates and to water soluble, readily excreted products. Biotransformation varies according to many factors such as species, strain, age, weight, emotional state, gut bacteria and sex.

^v The molecular specificity of enzymes is determined by mechanical fitting (shape), electrostatic and dipolar interactions, as well as electron and proton tunneling.

3.6.1.1. With Metabolic Rate

For example, **warfarin** is used both as a rat poison and as an anticoagulant, and is primarily broken down by CYP2C9 (F3.23). 18 % of people have a variant of CYP2C9 that slows their metabolism of the drug. They are more susceptible to side-effects, such as severe internal bleeding. In this case, slower metabolism leads to increased effects because the AUC is larger.

Rodents metabolize drugs more rapidly than people do. To reach the same blood level in a rodent model, administered doses are increased. Although the blood levels are then comparable, gastrointestinal tract and liver levels become much higher in the rat, which increases tumors in the liver and digestive system. In this case, faster metabolism leads to increased effects.

3.6.1.2. With Sex

In rodents, males have a capacity for some bio-transformations about 5 times that of females. This often corresponds to major response variations in animal experiments.

An example of a sex-based variation is the effect of the phyto-estrogen diadzein in rats. Injections increase growth hormone, testosterone and muscle mass in males, while the opposite happens in females¹⁰.

T3.19. Genes Expressed differently between the Sexes

Mouse Tissue	0 to 20% difference	300% difference
Liver	72 %	0.5 %
Fat	68 %	0.3 %
Muscle	55 %	0.1 %
Brain	14 %	0.1 %

Many of the sex differences in gene expression in the table above are hormone dependant: the differences are considerably attenuated if testes and ovaries are removed¹².

3.6.1.3. With Route of Administration

The toxic reaction is also influenced by the route of administration, as this determines the order in which the toxicant will encounter various organs, and the toxicant flow.

3.6.1.4. With previous Exposures

The biotransformation activity can be enhanced (*up-regulated*) by exposure to compounds such as phenobarbital, a technique used to harvest large quantities of the enzymes.

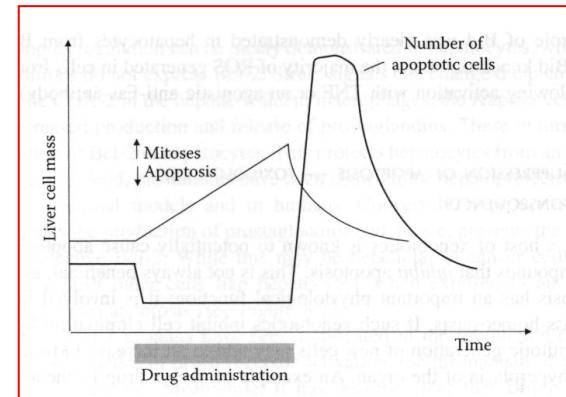
Not only will individual liver cells have more enzymes under phenobarbital induction, but the overall liver mass will increase with chronic exposure (F3.20).

Receptors such as Aryl Hydrocarbon Receptor (AHR), Pregnan X Receptor (PXR) and Constitutive Androstane Receptor (CAR) mediate the transcriptional responses that amplify the responses to xenobiotic exposures.

3.6.1.5. With and Within Species used

Rodents are frequently used as test animals, not because they are similar to humans, but because they are inexpensive, easy to house and breed, require minimal care and are easy to handle.

F3.20. Phenobarbital induces liver weight gain and enzyme production, and inhibits apoptosis in the liver. As the drug is removed, a sharp increase in apoptosis restores balance.
Boelsterli, 2007.



Commonly used test species *share* some of the metabolic capabilities of humans, but *lack* many others. These metabolic variations occur not only between species and strains, but between different individuals of the same species.

Even within a group of 75 inbred rats, the same high dose of acetaminophen (Tylenol) produces various degrees of liver damage. But pre-exposure analysis of urine makes it possible to predict with 85 % accuracy the level of liver damage that will be produced by the drug in individual rats¹¹.

Some differences can be traced to minor genetic variations among individuals, called single-nucleotide polymorphisms, or SNPs. Genome investigators at the National Institutes of Health (US) have already compiled more than 800,000 examples of such variations in humans.

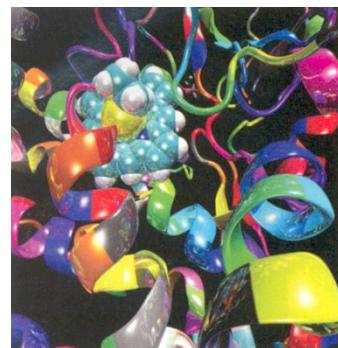
3.6.2. Phase-I Biotransformation

These **catabolic** (breakdown) enzymatic reactions deal with the insertion, addition or exposure of reactive groups, initiating the conversion of the molecule: a polar group is either unmasked or added, to enhance water solubility. The typical mechanisms used are **oxidation, reduction and hydrolysis**. The reactions are carried out by enzymes in microsomes and in the endoplasmic reticulum.

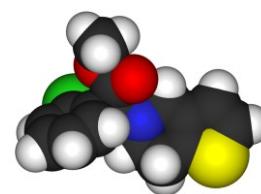
The major system is **cytochrome P-450** (F3.21, F3.23), an array of (7700 known distinct sequences) mono-oxygenase enzymes in the smooth endoplasmic reticulum. Fe⁺⁺ acts as a source of electrons and oxygen. The “450” (nm) refers to the bluish color of the enzymes.

The role of the **mono-oxygenases** is the oxidation or reduction of existing groups present on the molecule or the insertion of

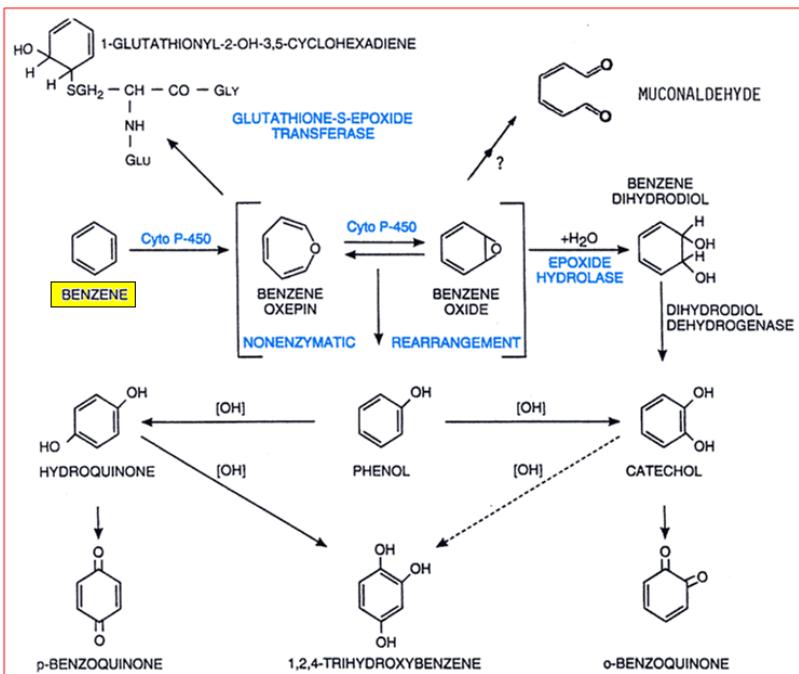
molecular oxygen into aromatic rings to form oxepin or benzene oxide (F3.22, center).



F3.21. Part of a CYP450 (multicolored ribbon) enzyme binding to putidaredoxin (light blue and white).
Center for Computational Research, U. of Buffalo.

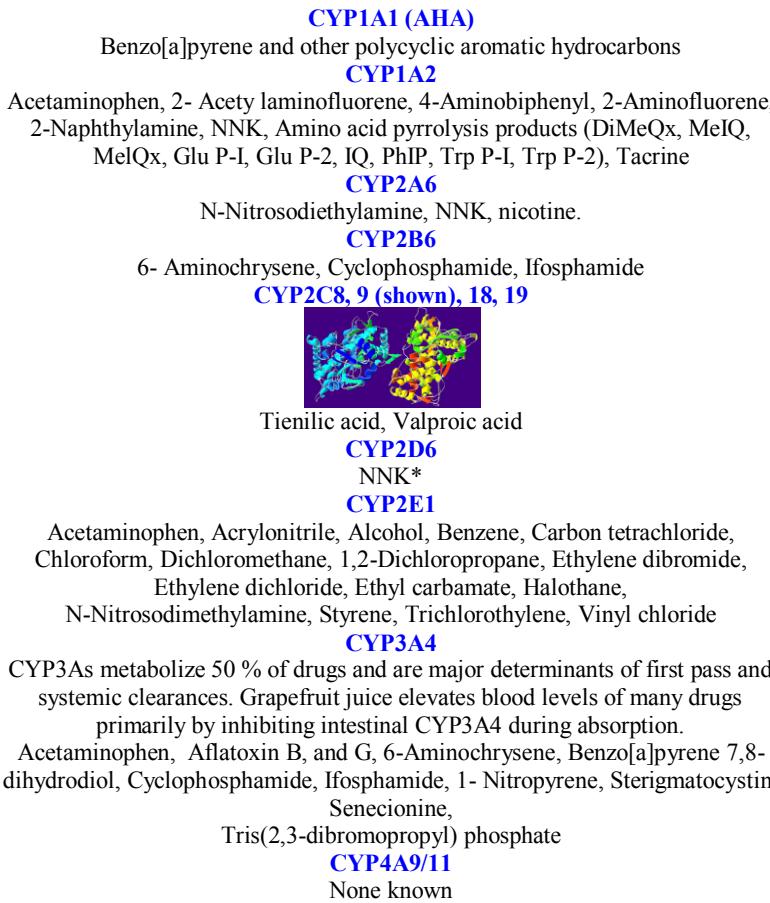


Clopidogrel inhibits blood clots and is prescribed after a heart attack to reduce subsequent coronary events. Clopidogrel is inactive, and must be metabolized in the liver by cytochrome P450 enzymes, including CYP2C19. Clopidogrel-treated patients who carry one or two ineffective variant alleles of CYP2C19 are 1.5 to 3.5 times more likely to die or experience cardiovascular-related complications than patients who carry high-functioning alleles. Genotyping would allow patients with these mutations to use alternate drugs^{13,14}.



F3.22. Metabolic pathways for benzene. Casarett & Doull.

NNK (nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone) is a tobacco-specific nitrosamine that requires metabolic activation by cytochrome P450 enzymes. Smokers who have two fully functional copies of the CYP2A6 gene smoke 7-10 more cigarettes per day than smokers who have only one. The enzyme CYP1A2 activates many cancer-causing chemicals in tobacco smoke. The enzyme is inhibited by drinking 500 ml of grapefruit juice every day, a tactic to reduce the chances of cancer from smoking.



F3.23. Xenobiotics activated to more toxic forms by individual CYtochrome P450 enzymes. The identification following “CYP” refers to enzymatic activity traceable to specific genes: class-subclass-gene. Growth hormone pulse profile* is a major determinant of which P450 enzymes are expressed. Casarett & Doull.

* Hormones are often most effective when released in **pulses** as opposed to continuous releases.

Another group of Phase I enzymes includes the **hydrolases** (esterases, proteases, lipases), their function being to hydrolyze peptide and ester bonds of a wide variety of chemicals to expose polar carboxyl, and hydroxyl or amino groups. These enzymes sometimes transform aromatic compounds into epoxides (R-O-R), compounds significantly more toxic (mutagenic) than their parents. There are many examples of xenobiotics activated by individual P450 enzymes to more toxic species, as shown in F3.23. On the other hand, CYP2B6, for example, also disables more than a dozen known herbicides, pesticides and industrial chemicals.

Many drug companies now conduct lab tests to determine the influence of potential drugs on common metabolic pathways, such as cytochrome P450 enzymes. If the drug affects them, it is pulled from further development, so that no interference with other drugs can occur.

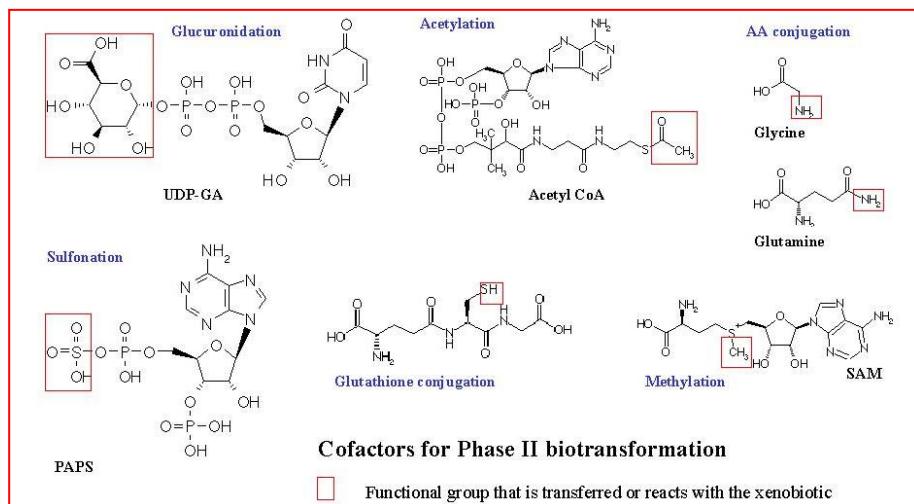
3.6.3. Phase-II Biotransformation

The introduction or exposure of reactive groups by Phase I prepares the way for Phase II. Although the enzymatic reactions also occur in microsomes and mitochondria, it is mostly cytosolic enzymes (F3.24) that **transfer covalently** normal body constituents (sulfate, glutathione, acetyl/glycine, methyl, glucuronyl, amino acids as well as ornithine) to reactive groups.

Note that a molecule must be *provided by the body* to the toxicant to further enhance hydrophilicity in these conjugation reactions.

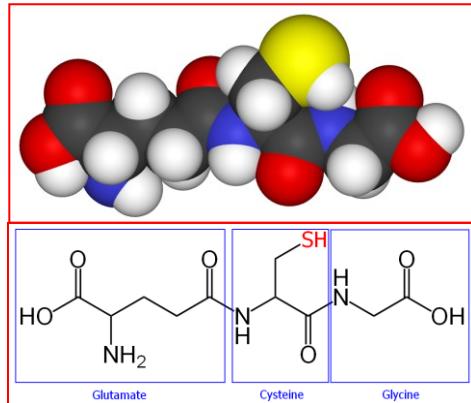
The conjugation converts the intermediates into water soluble products amenable to excretion via the urine and feces.

F3.24. PHASE II ENZYMES (“transfases”)			
DeTox Group	Enzyme Family	Enzymes	Substrates
Sulfate	SulfoTransferases	SULT 1A1 to 2B1	Phenol, Toluene, Acetaminophen
Glutathione	Glutathione S-Transferases	GST A1 to Z1	Peroxide, Acetaminophen, Heavy Metals
Acetyl	Arylamine, Aralkylamine or Glycine N-Acetyl Transferases	NAT 1 to 2, AANAT, GLYAT	Arylamine and Hydrazine drugs
Methyl	Methyl Transferases	COMT	Cathecols, Indolamines, Thiouracil
Glucoronyl	UDP-Glucuronosyl Transferases	UGT 1A1-2B28	Phenol, Trichloroethanol, Nicotinic acid
Amino Acid	Amino acid N-acyl Transferases	BACATs	CoA-activated Bile Acid
Ornithine	Ornithine Carbamoyl Transferases	OCT	Choline



3.6.3.1. Glutathione

Glutathione (shown) binds to heavy metals with its sulfhydryl (-SH) extremity. The transport proteins Multi-Drug Resistance Protein (MRP) and P-glycoprotein (Pgp) ship these adducts out of cells, and the body then eliminates the molecular complex through urine or bile.

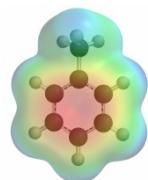


The conjugation of toluene derivatives with glutathione (F3.25) may result in the formation of nephrotoxic cysteinyl thioether derivatives (F3.25 - toluene conversion into benzyl sulfate, then into an N-acetyl-S-benzyl-L-cysteine derivative).

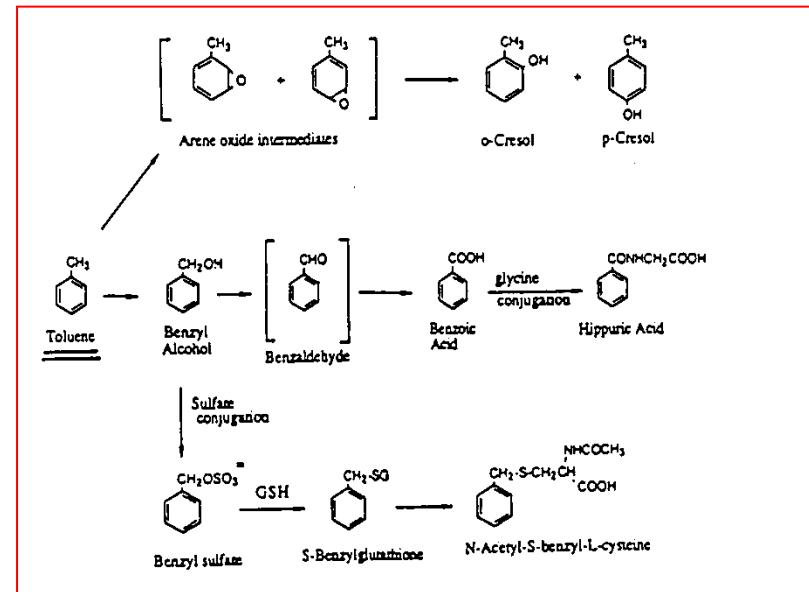
Free hydroxy groups, as in o-cresol or p-cresol formed from toluene, are susceptible to glucuronidation and sulfation.

Many chemicals already possess electrophilic groups (as in cresol or phenol, which have hydroxy groups) and do not need to undergo Phase I biotransformation, proceeding immediately to Phase II reactions.

3.6.3.2. Toluene



Toluene can be biotransformed into a number of products (F3.25), but not all of these may be produced by all species. Certain pathways may occur at low levels of exposure while, at high levels, a second or even a third pathway may



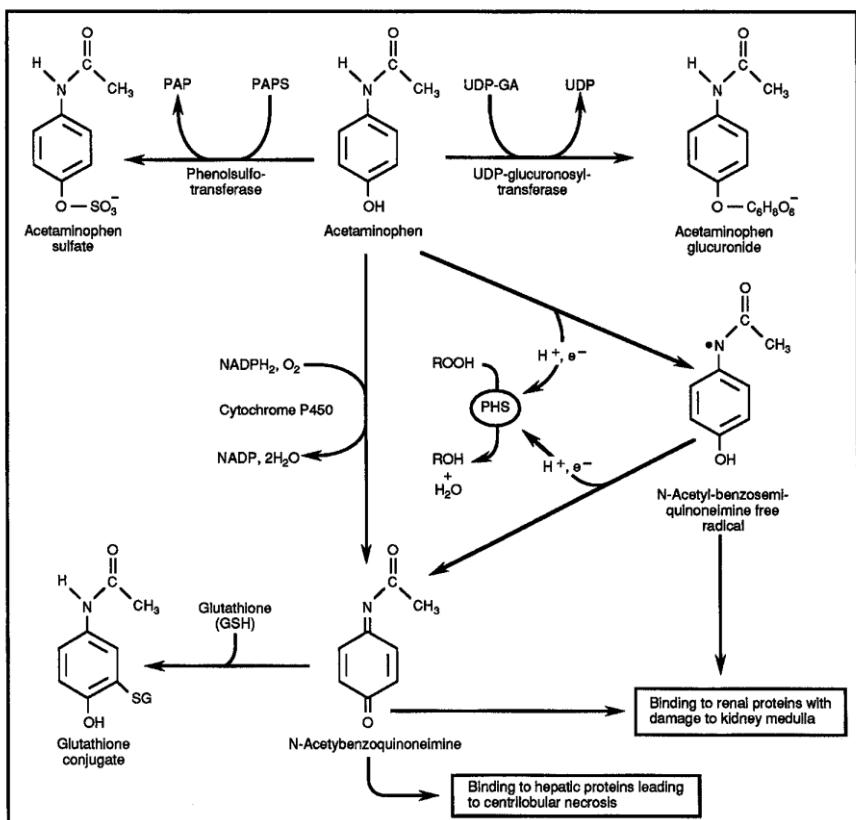
F3.25. Metabolic pathways for toluene.

Low et al. Toxicol. Indust. Health 4, 49-75, 1988.

become active. In any study of a chemical's excretion, it is advisable to know which excretory products are found at which dose, and have a method by which all can be quantitated. The rodents are notorious for introducing a new pathway when a threshold dose is attained.

3.6.3.3. Acetaminophen

Humans may do the same, as is shown in F3.26 for the drug acetaminophen. Acetaminophen ("Tylenol") is both hepatotoxic and nephrotoxic at high doses. The drug (F3.26, top-center) already has a handy hydroxyl group, useable to tag glucuronide or sulfate conjugates. However, these pathways can saturate at high exposures, because of enzyme limitations.



F3.26. Pathways of acetaminophen disposition.
(PHS = Prostaglandin H synthase).

With higher dosage, the cytochrome P-450 mono-oxygenases begin to react with the unconjugated drug, producing a quinoneimine derivative which can be utilized by the glutathione conjugating enzyme to form a mercapturic acid derivative. At excessively high dosage, this pathway can be saturated and even halted.

This leaves the reactive, electrophilic and unstable intermediate free to seek a "neutral" environment by reacting with any nucleophilic groups in the region - DNA, membrane proteins, cellular proteins, etc.

The result is cellular damage with leakage through damaged membranes and, if severe enough, necrosis and cell death.

3.6.3.4. Phase I and II as Rate-Limiting

In most tissues of the body, there are excess amounts of mono-oxygenases for the cells' needs, but the various conjugating enzymes are limited. Although sufficient for low level exposure, the rate-limiting step at high exposures is the activity of these enzymes and their dependency on

- (1) a sufficient cellular store of the conjugating substance, and
- (2) the rates of replacement or re-synthesis of these substances (glutathione, glucuronic acid, sulfate, glycine, etc.).

A dramatic decrease in the activity of the enzymes occurs until the stores are replaced, a period of 18 to 48 hr in many species. Repeated exposures result in repeated insults to the body's tissues and considerable damage if the reactive intermediates formed cannot be conjugated.

The formation of certain conjugates is quite different between species. For example, the human glucuronidates much of the acetaminophen ingested, with a small amount of sulfate formed unless the level of exposure is high, and then glutathione derivatives (leading to mercapturic acids) will be found. The guinea pig does the same. The rodents (mice, rats) produce quantities of both glucuronide, sulfate and glutathione derivatives.

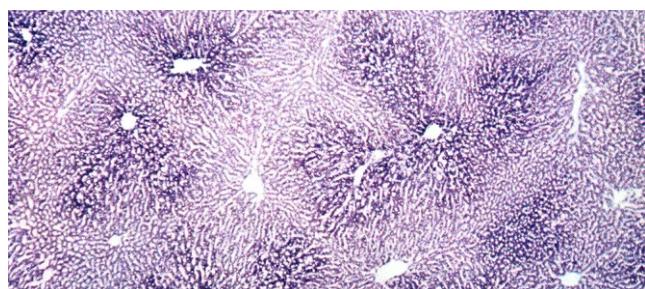
The conjugation of a simple molecule such as **phenol** is quite species-specific, as is shown in F3.28, showing the pathways of phenol and the oxidative hydroxylation product, quinol.

While Phase I and II enzyme complexes are ubiquitously distributed through the body's tissues, they are not *uniformly* distributed, but bear some relationship to the overall metabolic activity of the particular organ. Liver has the highest activity, the kidney has approximately 20 % of the liver's activity, while brain, muscle, etc. may have 1 to 10 %. This concentration of enzyme activity is responsible for chemical-induced cellular damage in the liver.

The detoxification properties of Phase II enzymes can be manipulated, even by simple dietary changes. Broccoli sprouts contain a substance, sulforaphane, which stimulates the production of Phase II enzymes, causing carcinogens to be more efficiently detoxified.

3.6.4. Toxicant Interactions

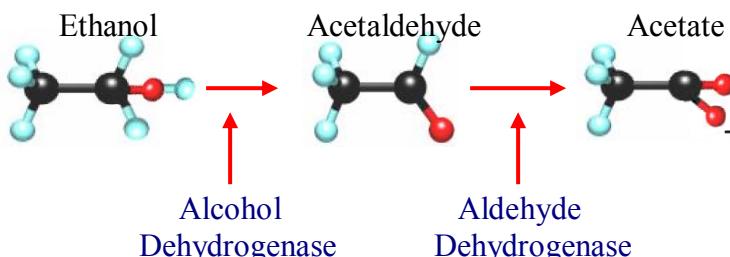
If more than one agent solicits the liver enzymes, interference or competition between two chemicals for biotransformation by the same or similar mono-oxygenases may result. The chemicals might be two components in a mixture or an "occupational" chemical plus an over-the-counter or prescription drug. Unfortunately, there are too few good, well studied, examples of toxicant interactions in the literature. However, three such studies are listed in the references²⁻⁴.



F3.27.
Distribution of
glucose-6-
phosphatase
enzyme in the
liver. X 25.
*Biological
structures, 1979.*

In the metabolism of **ethanol**, shown below, the enzyme alcohol dehydrogenase has an affinity for ethanol 100 times greater than to ethylene glycol.

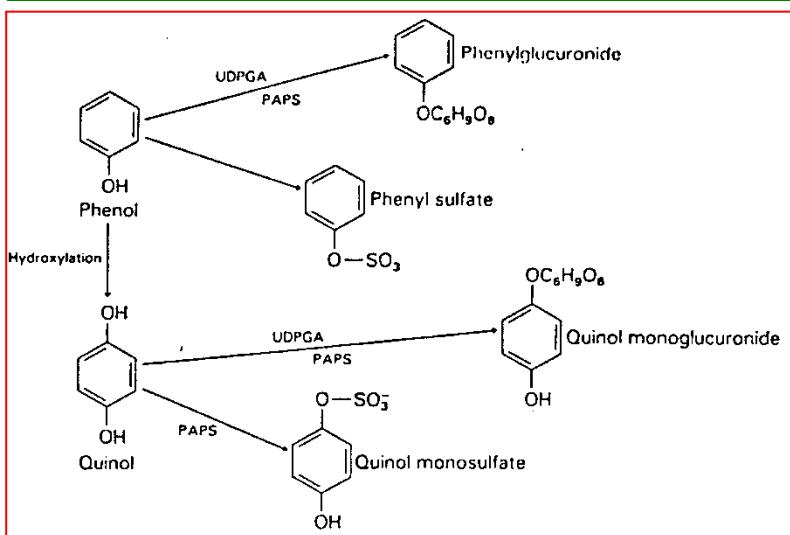
In case of ethylene glycol (car coolant) intoxication, intravenous alcohol is recommended. This allows ethanol to monopolize the enzyme, and for the renal excretion of unchanged ethylene glycol to occur slowly, avoiding its conversion to more toxic metabolites.



You might have noticed a slight trembling the day after alcohol intake. The enzymes monopolized for alcohol digestion would normally provide nervous system nutrients, but are not available in sufficient quantity to fill the needs of the nervous system.

F3.28. Species variation in the metabolic conversion of Phenol *in vivo*. Percent of 24-hour excretion as...

Species	Glucuronide		Sulfate	
	Phenol	Quinol	Phenol	Quinol
Pig	100	0	0	0
Indian fruit bat	90	0	10	0
Rhesus monkey	35	0	65	0
Cat	0	0	87	13
Human	23	7	71	0
Squirrel monkey	70	19	10	0
Rat tail monkey	65	21	14	0
Guinea pig	78	5	17	0
Hamster	50	25	25	0
Rat	25	7	68	0
Ferret	41	0	32	28
Rabbit	46	0	45	9
Gerbil	15	0	69	15



3.7. Excretion

3.7.1. Lungs

Lungs can breathe OUT toxicants as well as they can breathe them IN. For example, 90 % of alcohol is transformed into acetaldehyde in the liver, but 10 % is left unchanged, and can be exhaled or urinated (thus, the roadside test).

3.7.2. Liver

Feces can contain ingested toxicants which were not absorbed by the body at all, or toxicants that were secreted out either by the intestine or the bile (DDT and lead).

Toxicants that are being expelled through bile can be reabsorbed into the body, possibly to produce more damage to the liver. This re-absorption is called the **entero-hepatic loop**. Techniques have been developed to interrupt the loop by ingesting an adsorbent such as carbon black, that will bind tightly and carry toxicants into the feces.

Bile secretions contain detergent-like acids for digestion of fats in the intestine. Most of these acids are reabsorbed in the intestine. Lithocholic acid is not reabsorbed, and is one of the most toxic natural substances in the body, acting as a mutagen that also inhibits DNA repair, which has led in animal models to colon cancer⁶.

3.7.3. Urine and Feces

There are about 1 million nephrons in a kidney. The incoming blood loses fluids through the glomerulus by mechanical filtration (sieve) and proceed to recuperate needed components at the proximal tubule, the loop of Henle, and the distal tubule. The kidney is therefore an organ of RECUPERATION,

throwing out everything, and selectively reabsorbing precious components.

F3.29. Diagram of a nephron. *Medical Physiology, Guyton, 1981.*

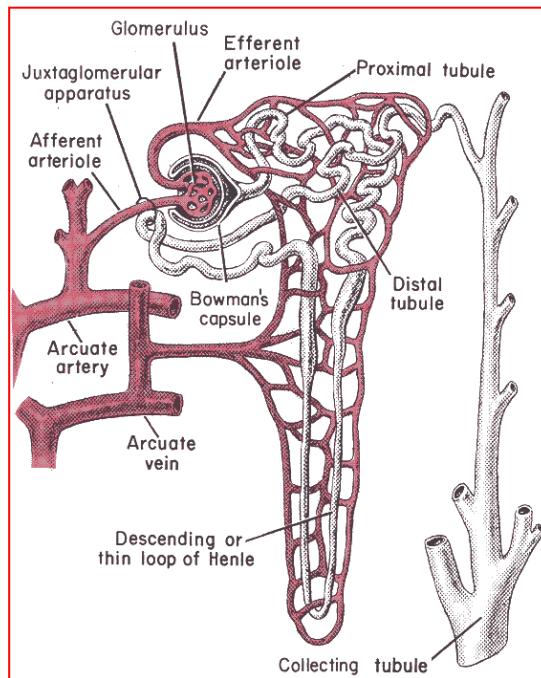
The strategy of making toxicants *hydrophilic* then becomes clearer. At the glomerulus, hydrostatic pressure forces small molecules smaller than 60,000 Dalton out into Bowman's capsule, thus conserving blood cells and large proteins. 20 % of all dissolved compounds less than protein size are initially lost in the kidney.

From the expelled fluid, 99 % of the needed components (for example, glucose) are recuperated in the loop of Henle. We would die rapidly without this constant recuperation.

Some active secretion into the urine occurs in the distal tubule, such that compounds that are actively secreted (PAH, Diodrast) can have a clearance greater than the glomerular filtration rate. The pH of the urine can vary between 4.5 to 8.

With this delicate anatomy, is it surprising that with a little hypertension, some protein or blood could leak into the urine?

The rate of elimination of many fat-soluble compounds can be enhanced by increasing the lipophilicity of



the gastro-intestinal tract content, for example by adding mineral oil to the diet.

Older people are known to be more sensitive to drugs. For example, they have increased sensitivity to central nervous system depressants, to side effects such as high blood pressure from psychotropic medication, and are more likely to experience hemorrhage from anti-coagulants. These changes correspond to alterations in the distribution and transport of drugs as a result of reduction in lean body mass (vs fat), serum albumin in the plasma, and total body water, as well as reductions in the function of organs which assist in the metabolism (enzymes) and elimination of substances.

3.7.4. Species Variations

Using benzene as an example, most animals appear to excrete low internal doses as metabolites in the urine. The urinary profiles in benzene-exposed mice, rats, and primates are shown in T3.30. For the low-dose treatments, a higher fraction of benzene is converted to hydroquinone and its conjugates in the mice compared to the rat. The urine of the monkeys, on the other hand, contains approximately the same fraction of hydroquinone as found in the urine of mice. One must keep in mind, however, that a smaller fraction of inhaled benzene is metabolized in the monkey compared to the mouse. The urinary profile of the chimpanzee is similar to that of the rat in regard to the hydroquinone fraction.

	Urinary metabolites (% of total)			6 hours at 5 ppm			6 hours at 50 ppm			6 hours at 600 ppm		
	Mouse	Rat	Monkey	Mouse	Rat	Monkey	Mouse	Rat	Monkey	Mouse	Rat	Monkey
Phenyl Conjugates	37	58	81	37	72	73	67	74	78			
Hydroquinone Conjugates	33	12	27	40	3	15	11	2	9			
Catechol Conjugates	na	na	8	na	na	8	na	na	9.9			
Pre-PhenylMercapturic Acid	6	10	na	1	11	na	15	17	na			
Muconic Acid	23	19	4.4	21	14	3.1	5	4	1.3			

F3.30. Urinary Excretion of (¹⁴C) Benzene. from Sabourin^{16,17,18}.

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Toxicokinetics

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4. Toxicokinetics

Toxicological science is not explicitly concerned with the difference between *Toxication* and *Intoxication*.

Toxication is the instantaneous toxic action resulting from the action of a toxic agent at a given moment in time. Whereas *Intoxication* refers to the effects of having been exposed to a toxic substance. So,

$$\text{In-toxication } (t) = \int_0^t \text{Toxication (Agent Concentration)} dt$$

This emphasis on *Intoxication* results from difficulties in the practical measurement of *Toxication*.

Usually, time effects in Toxicology are dealt with using indirect concepts such as categories of acute, sub-chronic and chronic effects.

Further, the evolution over time of the agent (primarily, agent concentration) is separated from the evolution of the organism itself.

Toxicokinetics is a quantitative description of the tissue levels of toxicants as a function of time in body compartments. It is concerned with peak blood levels, half-lives, body burdens and persistence of toxicants in organisms.

Toxicodynamics, a closely related field, investigates the changes in an organism as a result of toxicant exposure. Note that toxicodynamics also happens at the level of species, through evolution. Species change their design as a result of toxicant exposure.

2.5 billions years ago, there was no oxygen in Earth's atmosphere, and there was little in the oceans until 1 billion

years ago. It arrived after the proliferation of cyanobacteria. People did not invent pollution...it has been with us forever. Living systems adapted to a point by, for example, integrating mitochondria into their design. A shorter-term example is the prevalence of hemoglobin S in Africa to counter malaria. Extremely fast toxicodynamics is the development of drug resistance in bacteria. Humans are adapted to their toxic environment, but there is no doubt that the "design" can be improved⁶.

In an animal model with null toxicodynamics, the animal would not change as a result of intoxication. In this (unlikely) case, it would be possible for a single animal to be exposed to successively higher doses of a toxicant, and the experiments would yield the same results as if many animals were exposed to a range of single doses. In fact, the various manifestations of a toxicant could be uncovered by monotonously increasing the dose on a single animal.

Toxicokinetics quantifies absorption, distribution and elimination of an agent in an organism. It has the same basis as clinical pharmacokinetics², which monitors body burdens (blood and tissue levels) and therapeutic benefits.

The ideal Toxicokinetics for a drug (achieving the ideal plasma concentration) are not always easy to achieve. In the last 10 years, many oncology drugs have been approved which are in fact only innovations in drug delivery: getting the proper amount of medication into the proper compartment for the proper time interval.

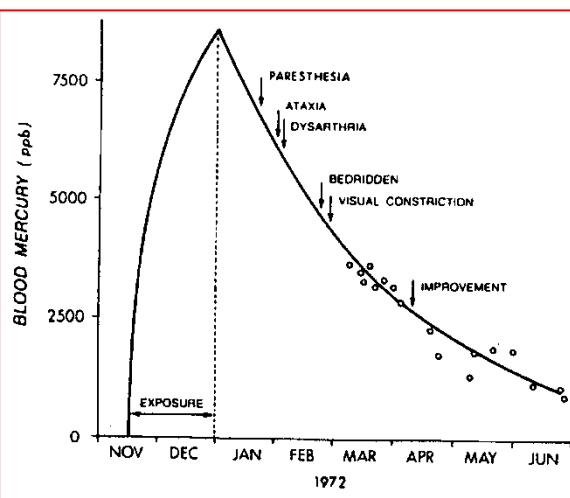
Toxicokinetics is concerned with the duration and intensity of toxic action by taking into account:

- ✚ the persistence of the toxicant and its metabolites in target organs,
- ✚ the accumulation of the agent in storage sites (body burden),
- ✚ the rate of elimination of the toxicant.

The *first step* in toxicokinetics is to divide the human body into

1. a central compartment (usually, the blood together with highly vascularized tissues), and
2. a certain number of peripheral **compartments** representing body segments with common properties (F4.2). The simplest model assigns to a single peripheral compartment the organs that are more poorly vascularized.

The *second step* of toxicokinetics is to make assumptions about the **rate of elimination** of the toxicant from the central compartment, specifically **zero order** or **first order** elimination (the models most commonly used).



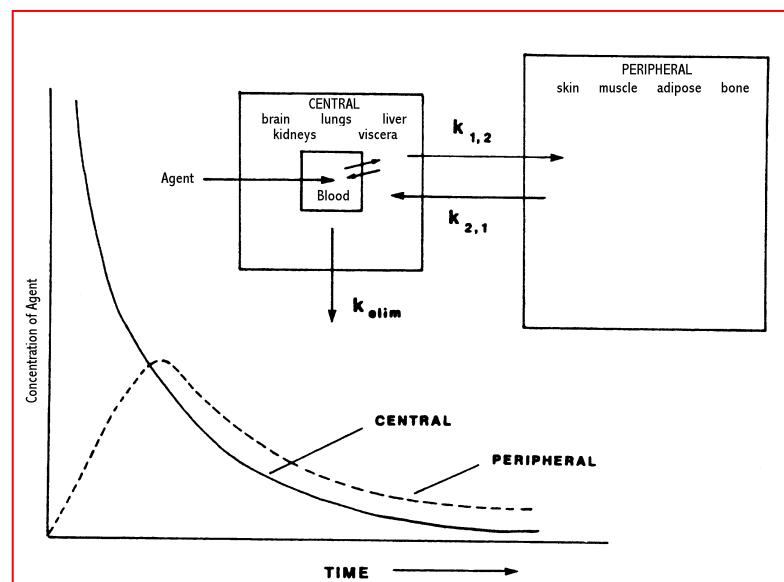
F4.1. Clinical symptoms in victims of methyl-mercury poisoning in the Iraq outbreak of 1971-72.
The solid line represents blood levels based on intake and the pharmacokinetics of methyl-mercury. Dots are actual observed blood concentrations.
Basis of Toxicity Testing, 1997.

Toxicokinetics is of great clinical usefulness in predicting the evolution of symptoms

in victims of intoxication. Optimal patient care needs quantification of toxicant concentration for many agents: acetaminophen, aspirin, ethylene glycol, methanol, ethanol, iron and other metals, lithium, carboxyhemoglobin and digoxin.

4.1. Notion of Compartment

A *compartment* is a part of the body with a uniform toxicant concentration. Examples of compartments: blood, plasma,

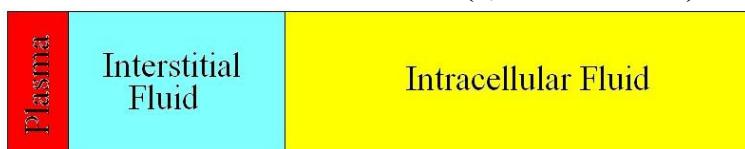


F4.2. Concentration of an agent in body compartments after injection into the blood (central square). Rapid equilibration between blood and Central compartment (short arrows) is already achieved before the beginning of the graph. The curves show equilibration between Central and Peripheral compartments, as well as elimination from the Central compartment. There is a route of escape from the central compartment only (k_{elim} , due to bladder, gastro-intestinal tract, lungs). There are rate constants to and from the peripheral compartment but no escape route, indicating that what goes out to the peripheral compartment (adipose tissue, bones) must be released back into the bloodstream for transfer to the central compartment.
Basis of Toxicity Testing, 1997.

stomach, bone, liver, extracellular fluid, mitochondrial volume, bound with albumin, etc. Almost anything can be a compartment, but the choice of modeled compartments depends on the properties of the toxicant.

Early studies used one or two "abstract" compartments (even if they had no physical reality) to describe the evolution of molecular concentrations over time. Now, the fashion is to use physiologically meaningful compartments of individual organs (intracellular, extracellular, alveolar space). Unfortunately, the basic data needed to set-up such models is often missing, partial or approximate.

BODY FLUID VOLUMES (8, 29 AND 63%)



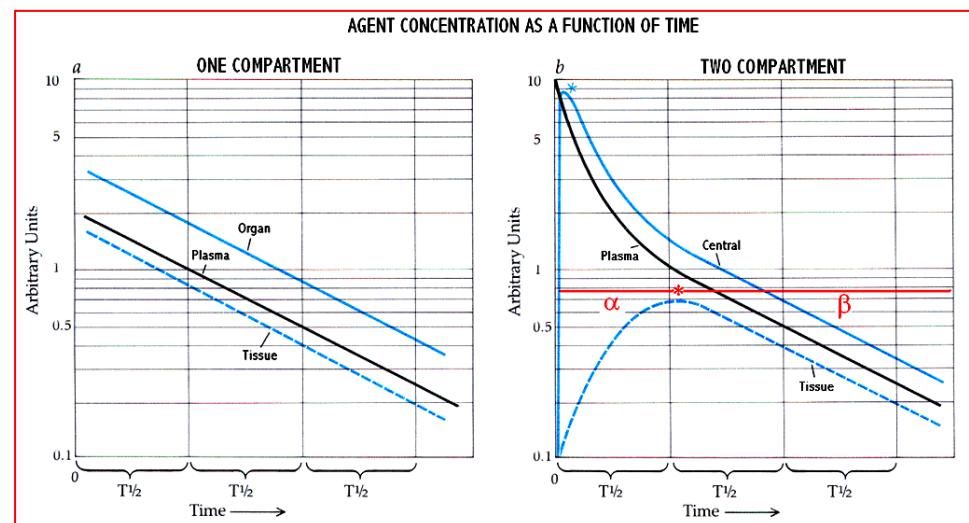
Although a compartment has a single concentration of a toxicant, the value usually drifts over time. An exposure generally results in the appearance of the agent in the bloodstream, but it usually does not remain there exclusively, as it is drawn out of the blood into other compartments. In F4.2, an agent injected into the blood is rapidly equalized in the **central** compartment made up of highly perfused tissues (this process is too fast to be shown in the graph). The blood level in the central compartment thereafter declines, indicating distribution into the **peripheral** compartment made up of the less vascularized tissues such as skin, muscle, adipose tissue and bone.

4.2. Theoretical Toxicokinetic Curves

Three important phenomena can be displayed in toxicokinetic curves (F4.3b): Absorption, Distribution ("α phase") and

Elimination ("β phase").

When a toxicant is eliminated from a single compartment model (F4.3a), one mostly *observes agent elimination* ("β phase") in the data. The absorption phase is vanishingly short, since the agent was administered directly into the blood, and there is no distribution, since there is only one



F4.3. Intravenous administration of a drug in a One- (a) and Two- (b) compartment model.

Modified from *Scientific American Medicine*.

compartment. The concentration differences between tissue, plasma and organ are due to the *partition coefficient* between the regions. Toxicokinetics based on blood analysis gives the investigator a way to predict future levels of the agent in the blood of the victim, but not in the target organs, unless the *partition coefficient* is known for all metabolites.

When elimination is from a two-compartment model (F4.3b), the toxicant rises instantly to its maximum value in plasma,

since it is injected. After reaching its peak in the blood (time 0), it is rapidly equilibrated between plasma and central compartment (blue asterisk).

After the peak concentration in the central compartment, the central and plasma variables are essentially the same, except for the influence of the *partition coefficient*.

Tissues, however, equilibrate more slowly, reaching a maximum later (red asterisk). After the tissue peak (red asterisk), all rates of decay are parallel. Before the red asterisk is the absorption and distribution phase, or α phase, and after the red asterisk is the elimination phase, or β phase.

The half-life of the drug changes during the α phase and is constant during the β phase.

The α phase may last for a few minutes, or for days or longer, depending on the speed of exchange of toxicant between plasma-central compartments and tissues.

Of interest to pharmacologists is whether the central concentration of a drug reaches a high enough value for a long enough period so that it is *efficacious*.

Elimination and some aspects of **biotransformation** can readily be measured by sampling urine, feces, exhaled air and carrying out chemical analyses for the agent and its biotransformation products. **Storage** cannot be measured conveniently, requiring invasive and frequently unpleasant techniques to secure appropriate samples.

In the case of heavy metals or halogenated hydrocarbons such as polychlorinated biphenyls (PCBs), the amount circulating in the bloodstream at any one time may represent only a small fraction of the body burden. 95% of lead body burden is stored in bone.

4.3. Elimination

Some biological fluids are available for sampling without using invasive techniques: exhaled air, urine, feces⁵. The data points on the right of curve of F4.3b correspond to pure elimination of the agent from the body.

The long-term excretion of a compound localized in a body storage site (adipose tissue, bone) is generally a slow process of equilibrium between the depot and the blood. Once the agent is in the blood (central compartment), metabolism and excretion (urinary, fecal, exhaled air) often occur, no elimination generally occurring from the peripheral compartment.

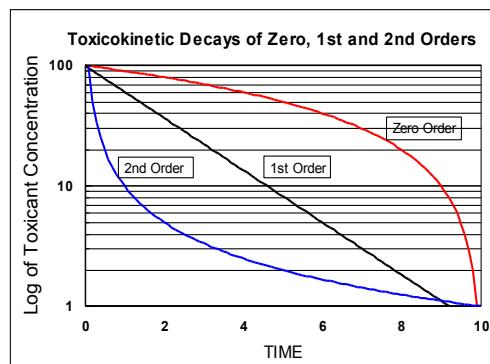
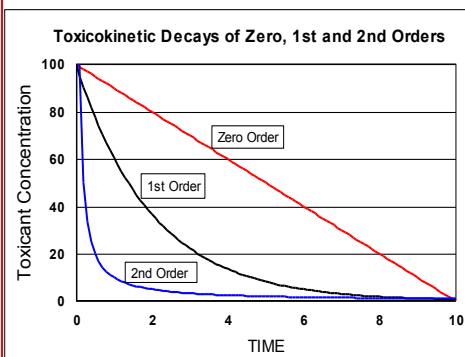
Examples of slowly eliminated compounds are PCBs, DDT and lead. When elimination of toxicants is very slow, elimination (particularly of heavy metals) can sometimes be improved by the administration of chelators: for example, the slow elimination of lead can be accelerated by EDTA chelation (T4.4).

T4.4. Chelators of Heavy Metals	
Metal	Chelator
Arsenic	Meso, 2,3-dimercapto-succinic acid (DMSA) 2,3-dimercapto-1-propanesulfonic acid (DMPS) Dimercaprol (BAL) D-penicillamine (PCN) N-acetyl-L-cysteine (NAC)
Cobalt	Ethylenediamine-tetra-acetic acid (EDTA)
Copper	DMSA, DMPS, PCN, Trentine dihydrochloride (Trien)
Iron	Deferoxamine (DFO), Deferiprone (Li)
Lead	DMSA, EDTA, BAL, PCN
Mercury	DMPS, DMSA, BAL, PCN

Another prominent chelator is DMSA. 20% of orally administered DMSA (10 mg per kg every eight hours for five

days) is absorbed from the gastrointestinal tract. One of the sulphydryls in DMSA binds to a cysteine molecule on albumin, leaving the other S-H to chelate metals. DMSA produces the best urinary excretion of mercury, greatest in the first eight to 24 hours after ingestion, and is effective at removing mercury from the blood, liver, brain, spleen, lungs, large intestine, skeletal muscle and bone. DMSA has removed two-thirds of the brain mercury deposits in animal studies. Quantitatively, elimination of the toxicant may proceed by various methods:

- (0) a fixed weight of the toxicant per unit time. This is “zero-order” or *saturation kinetics*, because the exponent of Toxicant concentration = 0,
- (1) a fixed percentage of the central compartment content per unit time, or so-called “first order”,
- (2) or the elimination can be even higher order (concentration squared is pictured in F4.5), reflecting facilitated elimination at higher toxicant concentrations.



F4.5. Zero-order and First-order decays are most frequent in toxicity studies, First-order usually succeeding Zero-order. By converting concentrations ($\mu\text{g}/\text{ml}$) into logarithmic values, the curves at right are obtained from the curves at left. In the log graph at right, Zero-order decay is curvilinear, and First-order decay linear.

4.3.1. Mathematical forms of Elimination

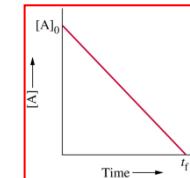
(k_x are the Elimination Rate constants; [] denotes a concentration)

$$\text{Elimin. rate} = k_0 [\text{Toxicant}]^0 + k_1 [\text{Toxicant}]^1 + k_2 [\text{Toxicant}]^2 + \dots \text{ (mg/hour)}$$

Let us consider 3 particular cases of elimination...

ZERO-ORDER: If k_1 and $k_2 = 0$,

$$\text{Elimination rate} = k_0 \text{ (mg/hour)}$$



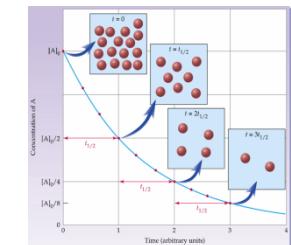
The elimination per unit time is then simply a constant equal to k_0 , that is a fixed amount of compound is eliminated for each unit of time. Elimination is independent of toxicant concentration, since the exponent of [Tox] is 0.

FIRST-ORDER: If k_0 and $k_2 = 0$,

$$\text{Elimination rate} = k_1 [\text{Toxicant}] \text{ (mg/hour)}$$

The elimination rate is proportional to the first power of toxicant concentration, ie $[\text{Tox}]^1$.

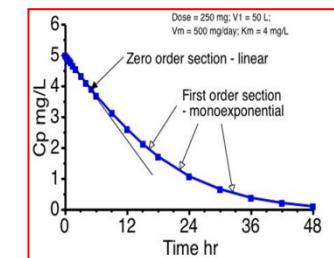
The concentration of toxicant is proportional to the elimination rate.



ZERO and FIRST-ORDER: If k_2 and higher terms = 0,

$$\text{Elimination rate} = k_0 + k_1 [\text{Toxicant}] \text{ (mg/hour)}$$

Many processes are first detected as “zero-order”, and then morph into “first-order” as time passes and toxicant concentration decreases (F4.8).



4.3.2. The Mathematics of First-Order Elimination

If the rate of elimination of a toxicant from the body is directly proportional to its concentration, this is expressed mathematically as the “linear first-order homogenous differential equation” shown below.

The derivative (d) of $[Tox]$ with respect to time (dt) is equal to a constant (k) times the concentration itself $[Tox]$.

$$\frac{d[Tox]}{dt} = -k [Tox].$$

“ k ” determines the speed of elimination (relative change in toxicant concentration per unit time and concentration), the minus sign in front of it signals a decrease in concentration with time. By moving factors in the equation above and integrating, we obtain

$$\int \frac{d[Tox]}{[Tox]} = \int -k dt, \text{ or } \ln [Tox_t] - \ln [Tox_0] = -k t.$$

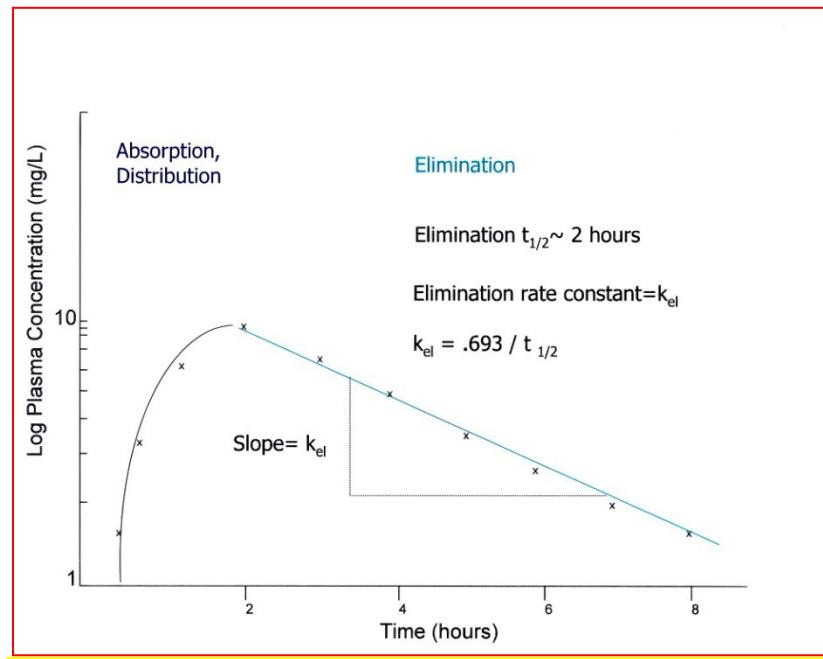
The equation above allows us to find the concentration of the toxicant at any time, $[Tox_t]$, if we know what the concentration was at $t = 0$, $[Tox_0]$.

If we prefer to use *common* (base 10, log), rather than *natural* (base e, ln) logarithms, the equation transforms as

$$\log_{10} [Tox_t] - \log_{10} [Tox_0] = -k t / 2.303$$

$$\frac{\log_{10} [Tox_t] - \log_{10} [Tox_0]}{t} = -\frac{k}{2.303}$$

The expression above indicates that if we plot the log of toxicant concentration, then the fall in concentration between time 0 and time t divided by the elapsed time t is equal to a constant.



F4.6. Half-life [$t_{1/2}$ or $\beta_{1/2}$] determination in the β -phase.

Graphically, this means that the slope of the curve (term at left) is negative (decreasing values of concentration) and is a constant ($k/2.303$), yielding a straight line, such as at the right of F4.6.

4.3.3. Half-Life Determination

Half-Life can be evaluated *only* during First-order elimination or beta (β)-phase, as shown in F4.6. A value, known as the β -half-life ($\beta_{1/2}$) can be found from the slope of the straight blue segment. The $\beta_{1/2}$ is the *time required to deplete the compartment (usually the blood) of 50 percent of the agent*.

If we assume that the concentration [Tox] is reduced in half at the half-life, the following equation holds:

$$\frac{\log_{10}\left[\frac{Tox_0}{2}\right] - \log_{10}[Tox_0]}{\beta_{\frac{1}{2}}} = -\frac{k}{2.303}, \text{ or}$$

$$\beta_{\frac{1}{2}} = -\frac{2.303 \left(\log_{10}\left[\frac{Tox_0}{2}\right] - \log_{10}[Tox_0] \right)}{k}, \text{ or}$$

$$\beta_{\frac{1}{2}} = -\frac{2.303 (-\log_{10} 2)}{k}, \text{ or}$$

$$\beta_{\frac{1}{2}} = \frac{0.693}{k}.$$

This equation relates the observed half-life to the elimination constant, k.

In practice, using experimental data, the half-life of a compound can be found from any two points ("1" and "2") which are part of a straight line on a log-linear curve.

$$\text{Slope on log-linear graph} = \frac{\log[Tox_2] - \log[Tox_1]}{t_2 - t_1} \quad (1)$$

[Tox₁] and [Tox₂] represent blood concentrations and t₁, t₂ the times at which these concentrations were observed.

Applying the above formula to the specific time interval t₂ which follows t₁ by one half life [β_½] and reduces [Tox₁] to half its value:

$$\text{Slope over one half-life} = \frac{\log \left[\frac{Tox_1}{2} \right] - \log[Tox_1]}{t_1 + \beta_{\frac{1}{2}} - t_1} = \frac{-\log 2}{\beta_{\frac{1}{2}}} \quad (2)$$

To find the numerical value of β_½, we need to equate expression (1) (with points 1 and 2 chosen on the straight segment on the curve) to the rightmost part of expression (2), as shown below:

$$\beta_{\frac{1}{2}} = \frac{-\log 2 \times (t_2 - t_1)}{\log[Tox_2] - \log[Tox_1]} \quad \text{or}$$

$$\beta_{\frac{1}{2}} = \frac{-0.301 \times (t_2 - t_1)}{\log[Tox_2] - \log[Tox_1]}.$$

Log-linear tracings are indispensable for easy visualization of elimination rates. Such tracings are obtained through plotting software or, if manual plotting is preferred, log-linear paper. Such paper can be generated through a printer by software (Graph Paper Printer 4.21 for Windows, [DVD Drive:\Graph Paper Program](#)) or by using the sheets supplied at the end of this text.

The formalism above should not obscure the fact that half-lives can usually be found easily by simple curve inspection on a log-linear graph, or by simply inspecting the values of a table...

Follow these steps:

1. Delimit a straight segment on the log-linear curve.
2. On this segment, find two concentrations that are a factor of 2 apart (ie, 10 and 5).
3. The time interval between these two concentrations is the half-life.

This simple method should be used to verify calculations... Since many compounds can be stored effectively in relatively inaccessible body compartments with an exceedingly slow

release of the agent, half-lives in humans can be years. For example the PCBs stored in our fat have elimination times that increase as the PCB concentrations decreases. Even if our exposure stopped tomorrow, we would all still die with a measurable body burden of PCBs.

4.3.4. Clearance

A good example of a first-order elimination concept often used in medicine is **clearance**. Clearance in ml/min is the volume of fluid that is totally cleared of the toxicant per minute.

If a chemical is not reabsorbed at all by the kidney, the **clearance is equal to the glomerular filtration rate**.

This is true of inulin and mannitol, used as test substances for glomerular filtration rate determinations.

If the substance is actively secreted, the clearance can be greater than the glomerular filtration rate.

A convenience of *clearance* is that it is additive between organs such as kidneys, liver, intestine, etc...

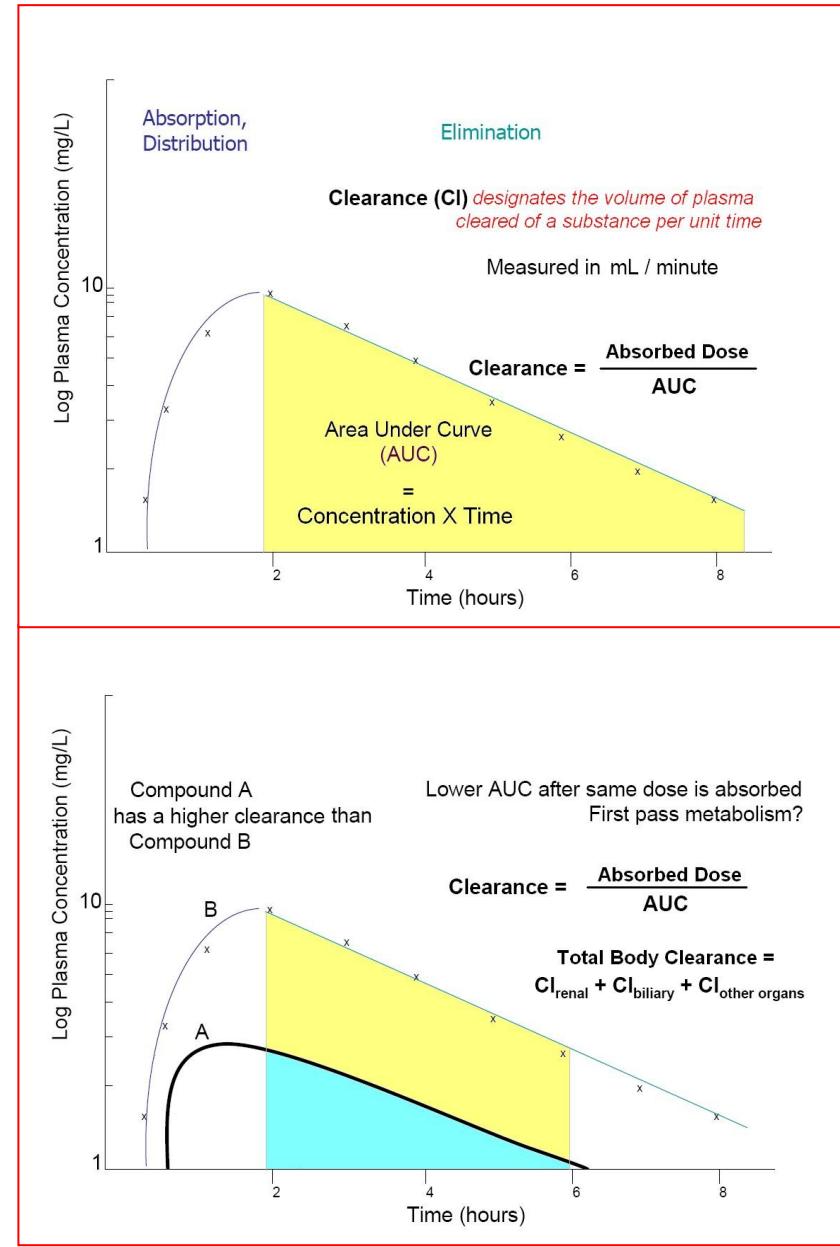
Clearance is of critical importance in recognizing kidney diseases in the clinic.

F4.7 (lower) shows how two compounds administered in the same amount can have their clearance determined by continuous measurement of the plasma concentration of the substance.

4.4. Analysis of Toxicokinetic Curves

4.4.1. Summary Characteristics of Zero-Order Elimination

1. Rate of elimination is a constant, independent of the amount of toxicant present in the body.
2. Quantity of Toxicant in the tissues decreases by a constant amount per unit time (mg/hour).
3. *Linear plot* of Toxicant concentration yields a straight line.



F4.7. Practical determination of clearance.

4. Toxicant half-life concept is inapplicable.

4.4.2. Summary Characteristics of First-Order Elimination

1. Rate of elimination is proportional to the amount of toxicant present in the body.
2. Quantity of Toxicant in the tissues decreases by a constant fraction per unit time (% / hour).
3. *Semi-log plot* of Toxicant concentration yields a straight line.
4. Toxicant half-life is independent of the dose.

An elimination process limited by bio-synthesis (such as Phase II biotransformation), may be **0-order** if the conjugation product is produced by the body at a fixed rate. The liver has a limited ability to synthesize the needed molecules, and excretion can only proceed after conjugation.

Often, a zero-order elimination reflects a limit in liver bio-synthesis or in the kidney's active secretion capability.

However, if a process is limited by *diffusion*, one might expect **first-order** kinetics, since a higher concentration gradient of the agent will result in more toxicant transfer and in more toxicant being processed, assuming no other limits.

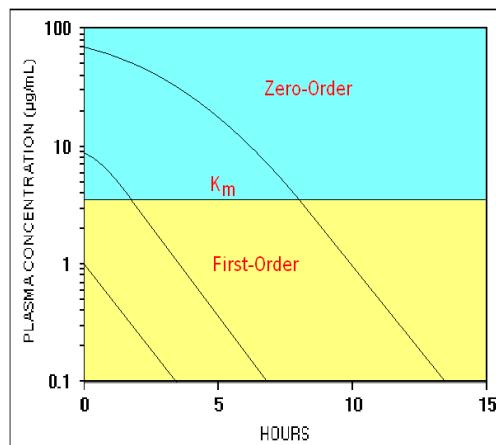
Elimination in the kidney is typical of such a process. The more toxicant enters the glomerulus, the more will be carried away into the urine, assuming no further transport of the agent across membranes.

Therefore, a first order elimination often reflects a process of elimination governed by passive elimination by the kidneys.

Many elimination curves are first "zero-order" and then morph into "first-order", as time passes and toxicant

concentrations decrease.

This is explained as follows. In the initial stages of high level intoxications, stored reserves of conjugation products are used very rapidly, thereafter leaving the elimination process to work only with conjugation products that must be synthesized *de novo*. This limits the speed of elimination to a zero-order process. As concentrations diminish, conjugation products again become plentiful and diffusion, rather than conjugation product availability, becomes limiting.



F4.8. Zero to first order kinetics.

The transition from zero-order to first-order occurs at the *Michaelis-Menten constant concentration (K_M)*, which is defined as *the toxicant concentration at which the velocity of an enzyme reaction is half the maximal velocity*.

- ⊕ When the concentration of a chemical in the body is **higher than K_M** , the rate of elimination is no longer proportional to concentration (**Zero-Order**).
- ⊕ When the concentration of a chemical in the body is **smaller than K_M** , the rate of elimination is proportional to concentration (**First-Order**).

4.5. Toxicodynamics: drifting Elimination with Dose

A further complexity is introduced in the process of elimination by the fact that the *elimination rate* in the beta phase can change with the dose administered.

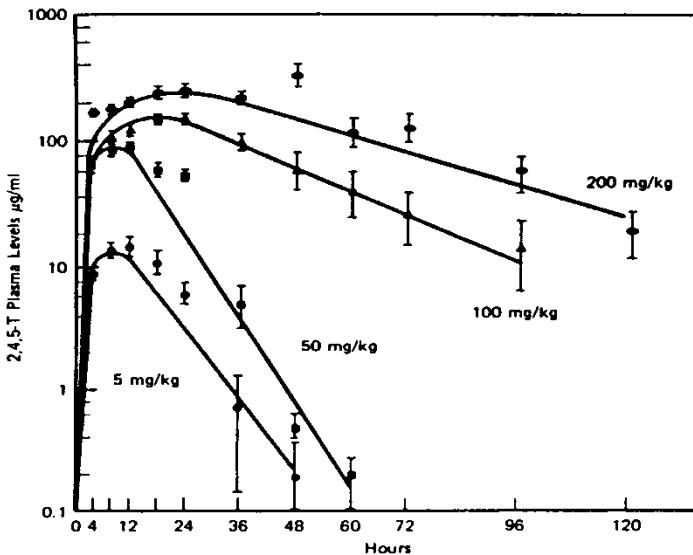
The same agent can display different half-lives at various doses, as shown below for the herbicide, 2,4,5-T.

2,4,5-T is a highly polar organic anion that does not undergo extensive storage, is not bio-transformed in the body, but is eliminated via the kidney by both glomerular filtration (the major route) and active secretion through the cells in the proximal tubule of the nephron.

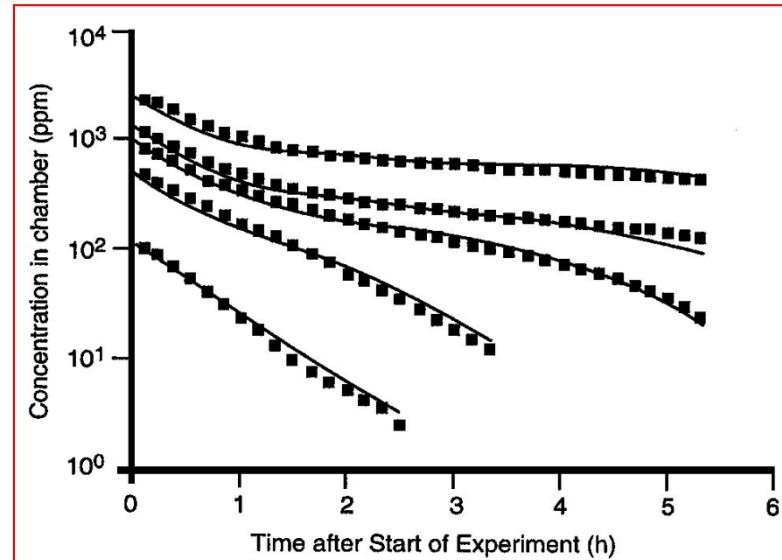
Essentially, the straight curve segments show urine excretion, with only one parameter of the β -phase (elimination) in operation.

The fact that at a given concentration in the blood, the half-life of the chemical can show different values (slopes in F4.9) indicates that the elimination process is altered by the dose administered (*Toxicodynamics*). Therefore, the *elimination performance of the organism was altered by the drug*.

A particularly ingenious experiments is shown in F4.10 below, where 3 rats are left to metabolize chloroform (CHCl_3) inside a closed cavity. The “biomonitoring” is achieved by simply measuring the concentration of chloroform in the atmosphere of the cavity. As the dose of chloroform increases in various separate experiments, we can see the transition from First-Order to Zero order elimination, reflecting a likely saturation of Phase II synthesis.



F4.9. Plasma 2,4,5-T of rats following various single oral doses.
Basis of Toxicity Testing, 1997.



F4.10. Disappearance of chloroform from a chamber containing three rats.

4.6. Multiple Compartments

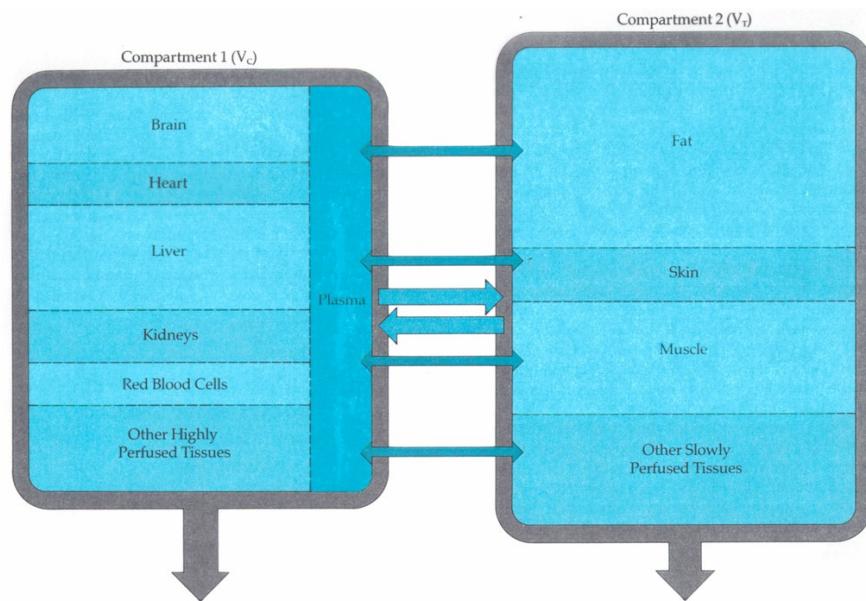
A **one** compartment model is typical of the case when blood and body organs reach equilibrium rapidly between them for toxicant concentration: a single compartment is therefore representative of the whole body, and excretion proceeds from this single compartment.

In many cases (such a lead, or any lipophilic chemical), a single compartment is not enough, and one must resort to two or multiple compartments, with elimination proceeding (in most implementations) from a central compartment that is dynamically connected for toxicant exchange with other compartments, as shown in F4.11.

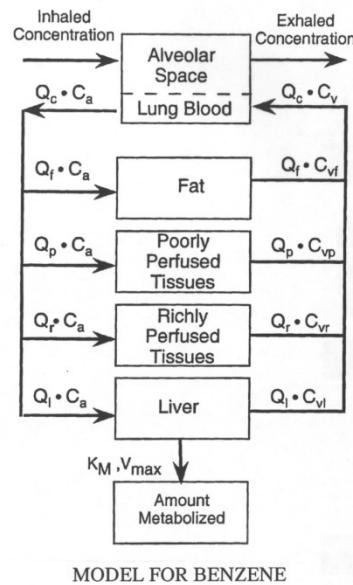
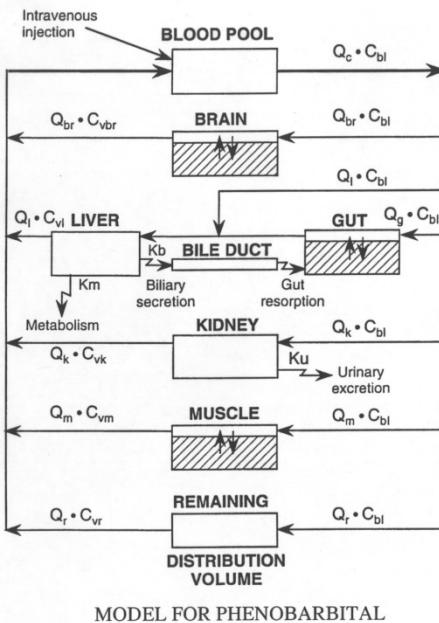
Toxicokinetic computerized models can become very complex with a multitude of compartments, as shown in F4.12. But models of such complexity usually have limited usefulness "in the field"¹.

Calcium illustrates the importance of compartments. Living cells maintain an intracellular environment that is markedly different from the surroundings. Sodium chloride, abundant outside the cell, is poisonous inside the cell. *But few things are as poisonous as calcium:* molecule for molecule, it is many times more poisonous than cyanide. Both sodium and calcium are therefore vigorously pumped out of the cell. To eliminate calcium, many cells render it insoluble by combining it with carbon dioxide to form extracellular calcium carbonate. This can be discarded or used as armour, or various forms of structural support (i.e. a skeleton).

Many exposures are of the repeated (hourly, daily, weekly) or extended (years) variety, the level at any one time being insufficient to cause any overt toxicity. Successive exposures add to the body burden, with adverse effects perhaps appearing in the future.



F4.11. Two compartments, two elimination routes. V_c = Central Compartment (highly perfused, equilibrating within a few minutes), V_t = Tissue Compartment. Horizontal arrows are individual rate constants between plasma and tissues. Tissues in compartment 2 reach equilibrium with plasma at different rates, but these are sufficiently similar to be modeled by a single constant (pair of larger horizontal arrows). The surface of the tissues in this representation corresponds to the amount of drug contained in this tissue. Vertical arrows show elimination from both compartments. *Scientific American Medicine.*



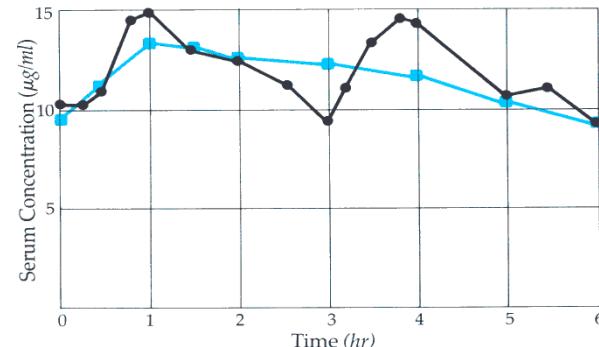
F4.12. Complex models for Phenobarbital and Benzene toxicity. Qs are blood flows (l/i/hr), Cs are concentrations (mg/l), Ks are elimination constants. Compartments that are diffusion-limited are separated in two, reflecting the fact that blood-borne movement of the toxicant is much faster than membrane crossing. *Casarett & Doull*.

4.7. Multiple Exposures

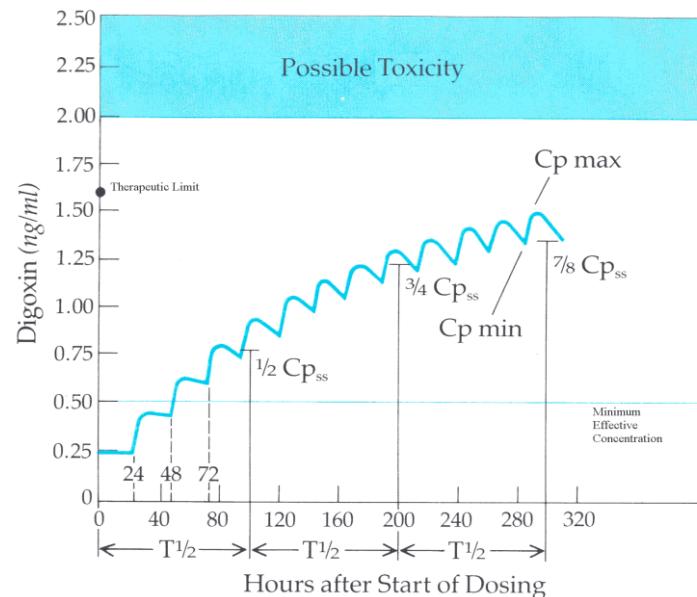
4.7.1. Repeated Exposure

Depending on the time interval between exposures, the subject may have had sufficient time to eliminate the earlier dose, but in many cases, there is a cumulation of the doses, leading to a climbing *body burden*.

As the concentration approaches the critical limit in the target locations, signs and symptoms of intoxication will appear in subjects. The signs and symptoms may reduce when people are protected from exposure, such as during the weekend.



F4.13. Repeated exposure. The black curve represents two administrations of the standard form of a drug, while the blue represents the slow-release form. Note the linear vertical axis. *Scientific American Medicine*.



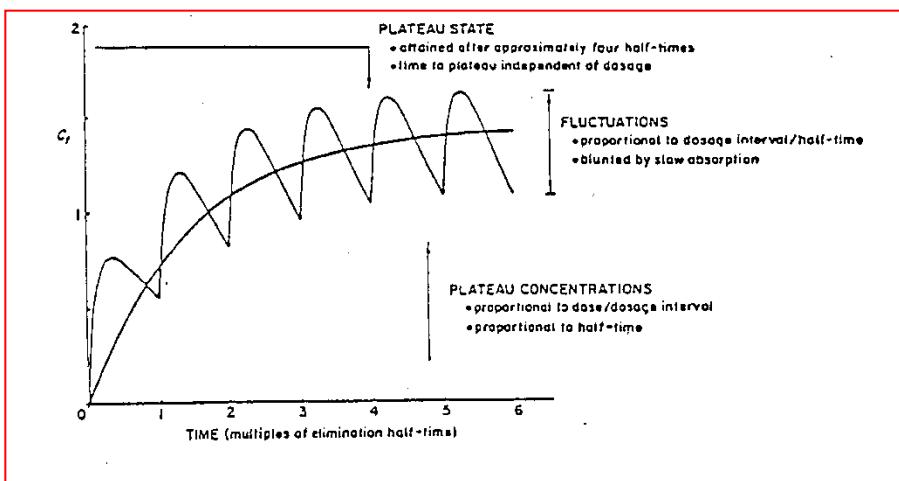
F4.14. Accumulation of body burden in the therapeutic administration of digoxin. A 0.125 mg digoxin tablet is administered daily to a 65-year old woman, 70 kg, treated for atrial flutter-fibrillation. Bioavailability is 0.65. Estimated half-life based on renal function is 100 hrs, and clearance is 2.1 liters/hr. Note the linear vertical axis. *Scientific American Medicine*.

4.7.1.1. Plateau

If one systematically exposed a subject to successive doses of an agent, retaining the assumptions that the β -half-life remains unchanged, the rising plasma concentrations would allow the subject to excrete more and more of the drug, until his excretion balances his intake.

After a certain number of exposures, we approach a "plateau", the blood concentration oscillating up and down during and after exposure but, within limits, remaining relatively constant.

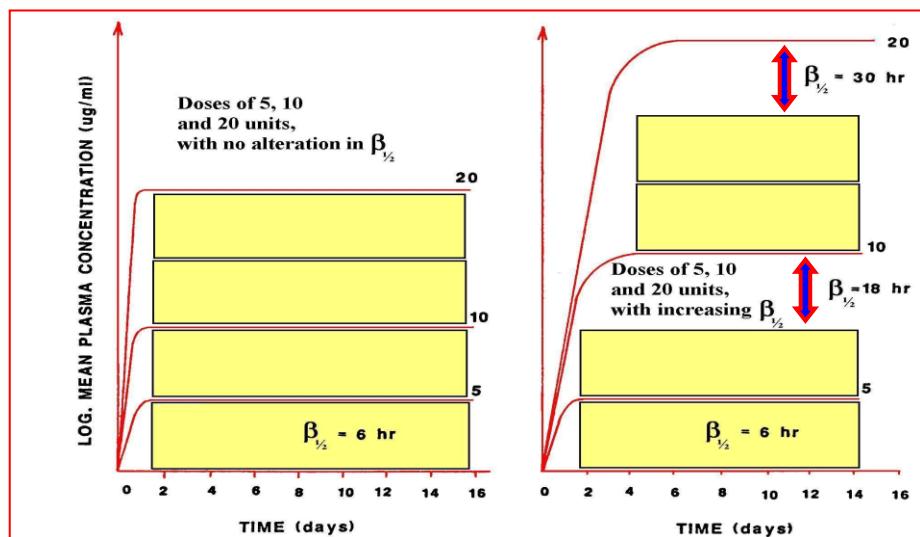
An exposure scenario familiar to physicians who want to obtain high therapeutic blood levels of an agent early is to give an initial large dose (bolus) followed by lower, *maintenance* dosage.



F4.15. Factors influencing blood concentrations under repeated identical exposures to toxicants. Assumes that the $\beta_{1/2}$ does not alter with increasing concentration of agent in the body. *Basis of Toxicity Testing, 1997.*

The TIME TO ATTAIN A PLATEAU in the blood concentration is determined as four to five half-lives of the agent (see F4.15). The half-life ($\beta_{1/2}$) is inversely proportional to the rate of elimination, but is normally independent of the level of the exposure.

The CONCENTRATION AT PLATEAU is dependent on the level of the exposure, the exposure interval and the $\beta_{1/2}$ (F4.15).



F4.16. Repeated exposures lead to plateaus of toxicant concentrations which are influenced by altered $\beta_{1/2}$.

Example: We consume some 50 micrograms of Cadmium in our food each day (ubiquitous in its environmental distribution). Cadmium has a biological half-life (not blood half-life) of approximately 7 years. If one determines body burdens of Cd, there is considerable variability until one reaches 35 years of age, at which time, if there is no additional

source of exposure (industrial), the body burden becomes relatively constant.

An illustration of the influence of $\beta_{1/2}$ in repeated exposures is shown in F4.16, where the time needed to attain maximum concentration in the blood, as well as the plateau reached are altered.

In F4.16 *left*, the organism reacts similarly in excretion performance, irrespective of the dose. Three different continuous exposures with a $\beta_{1/2}$ of 6 hours all attain their plateau at 24 hours, and the blood level attained at 20 exposure “units” is 4-fold higher than that at 5 “units”.

In F4.16 *right*, the organism excretes less effectively with increasing dose. As a result, $\beta_{1/2}$ increases (6, 18 and 30 hr) with the exposure level (5, 10, 20 units), and the time to attain the plateau shifts from 24 hr to 72 hr to 120 hr.

The stable blood concentrations attained are not “proportional” to dose (the gap is indicated by the blue-red arrows).

In workplace hygiene, toxicokinetic calculations may occasionally adjust the exposure standard (uniformly set for 8 hours) if unusual work schedules are involved. Calculation can be performed based on the number of hours worked each day, the number of hours worked each week, and the half-life of the agent. Adjustments are not required for agents with a half-life less than 3 hours (Short-Term Exposure and Ceiling Limits take over) or exceeding 400 hours⁷.

4.7.2. Simultaneous Exposures: N-in-1

Pharmaceutical companies looking for drugs with desired

pharmacokinetic parameters in animal models such as the rat, the dog and even some *in vitro* models (Caco-2 cell model) invest a lot of money obtaining concentration vs time profiles.

The *N-in-1* technique was introduced to reduce testing costs. It involves the oral or intravenous *co-administration of multiple drug candidates into a single model* and their separate analysis over time using gas chromatography and high performance liquid chromatography.

The *N-in-1* strategy is capable of significantly speeding determinations, using a fraction (1/N) of the animal resources. Data show pharmacodynamic profiles similar to those obtained for compounds in individual dosing.

Of course, compounds that can be converted to one another should not form part of a simultaneous pool. Even though there are some risks associated with the *N-in-1* method, they can be minimized by careful selection of the *N-in-1* pool.

4.8. Toxicokinetics Examples

4.8.1. Doping

The elimination of drugs from the blood and urine is a central variable in the evaluation of doping. In the table below, Drug Detection Times are expressed in terms of lower and upper boundaries. Many factors determine an individual's placement within these boundaries. In general, the following factors will increase a drug's detection time: chronic use, high levels of use, high drug potency, high urine pH, slow body metabolism, high body fat count (for Marijuana and PCP), low fluid intake, and overall poor health.

In the case of hexacarbon neuropathy, a certain body burden of n-hexane is required to produce the characteristic central and peripheral distal axonopathy, and the neuronal damage is advanced before the diagnosis can be confirmed.

Removal of the individual from the exposure site frequently results not in a lessening of the signs and symptoms, but in a worsening of the condition, much to the horror of the attending physician, who now doubts the accuracy of his diagnosis.

What should be appreciated is that it is not n-hexane that is toxic, but a biotransformation product. The body burden of the agent must be eliminated via this metabolic route, and so toxicity remains for about two weeks, during which the symptoms may remain stable or become more pronounced. They only begin to subside later. Depending upon the severity of the neurological damage, the patient may recover completely, make a partial recovery or remain permanently incapacitated.

T4.17. Drug Detection Times from Drug Test Success	
Alcohol	6 hours to 1 day
Amphetamines	1 to 4 days
Barbiturates	Short-acting (Allobarbital, Alphenal, Amobarbital, Aprobarbital, Butabarbital, Butalbital, Butethal, Pentobarbital, Secobarbital): 1 to 4 days Long-acting (Barbital, Phenobarbital): 2 to 3 weeks Short-term Therapeutic Use: 3 days
Benzodiazepines	Long-term Chronic Use: 4 to 6 weeks
Cocaine	2 to 5 days
LSD	1 to 4 days
Marijuana	Casual Use: up to 7 days Chronic Use: up to 30 days or longer Note: THC, Marijuana's primary psychoactive ingredient, is stored by the body in fatty lipid tissue. From there, it is slowly released into the bloodstream and urine for up to several weeks. Chronic users and individuals with high body fat count are at the greatest risk of long-term detection.
MDMA (Ecstasy)	1 to 4 days
Mescaline	1 to 4 days
Methadone	1 to 7 days
Methamphetamine	1 to 4 days
Methaqualone	up to 14 days
Nicotine	1 to 2 days
Opiates	1 to 4 days
PCP (Phencyclidine)	Casual Use: up to 7 days Chronic Use: up to 30 days Note: PCP is stored by the body in fatty lipid tissue. From there, it is slowly released into the bloodstream and urine for up to several weeks. Chronic users and individuals with high body fat count are at the greatest risk of long-term detection.
Propoxyphene	1 to 7 days
Psilocybin (Mushrooms)	1 to 3 days
Steroids (Anabolic)	Oral: 2 to 3 weeks Injected: 1 to 3 months Nandrolone: up to 9 months
Tricyclic Antidepressants	1 to 9 days

4.8.2. Examples of Clinical Toxicokinetics

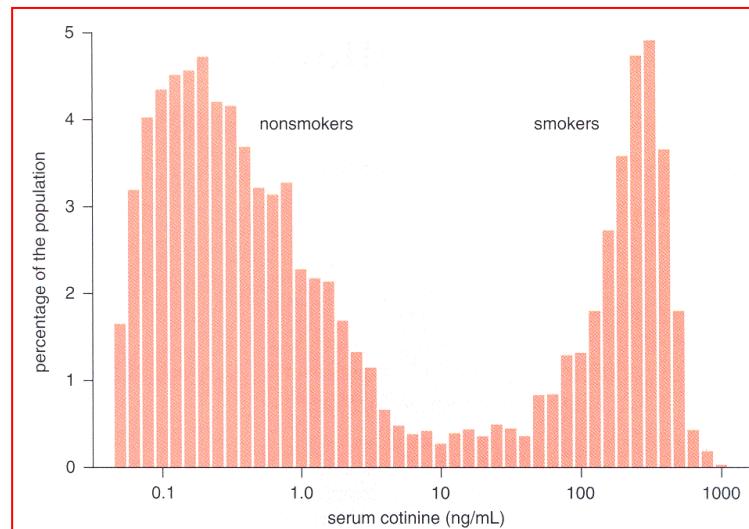
4.8.2.1. Toxicokinetics in the Blood and Urine

Toxicants or their metabolites can be sampled from body gases, body fluids or body tissues. Toxicants in exhaled gases are often detectable only for relatively short periods after intoxication, as is the case for alcohol. Therefore, one may need to be present on the spot for useful sampling. Because of this, and because of the discomfort of obtaining most body tissues (with the exception of nails and hair), sampling is often done using accessible body fluids, specifically urine and blood. Sensitive and specific biomarkers are available for metals, dioxins, furans, PCBs, pesticides, volatile organic compounds, phthalates, phytoestrogens and tobacco smoke.

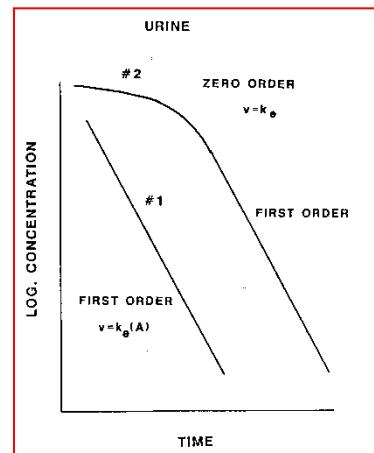
What classes of toxicants can we expect to find in either of them?

Dioxins, PCBs and organochloride pesticides reside for months or years in the body, either because they are metabolized slowly or because they are hidden in lipophilic (fatty) compartments. For example, the half-life of TCDD is 2–4 wks in rodents, while it is approximately 10 yrs humans. These properties tend to lower excretion rates.

Organophosphate pesticides, alcohol and volatile organic compounds, on the other hand, reside in the body only for hours or days, because they are metabolized quickly. They may be favorite targets of enzymes, or enzymes may have good access to them because they are not hidden in lipophilic compartments. This tends to heighten excretion rates.



F4.18. Exposure to tobacco smoke is measured as blood (serum) cotinine in this histogram. It clearly discriminates between smokers and non-smokers. *American Scientist, 2004*



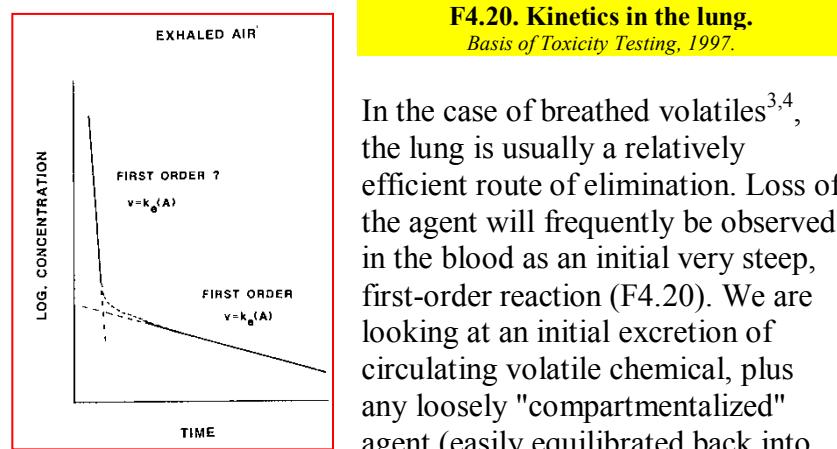
F4.19. Kinetics in urine.
Basis of Toxicity Testing, 1997.

Therefore, persistent chemicals are often measured in blood, while transient ones are generally measured in urine. Bio-transformation pattern is also a factor, some compounds (benzene) forming urinary metabolites, while others do not (dioxin).

F4.19 shows typical curves for urine. In #1 (lower exposure), a first-order linear relationship, where excretion half-life is dependent on an elimination constant (k_{elim}), not on the concentration of the chemical. In #2

(elevated exposure), the initial part of the relationship is curvilinear, but becomes linear with time. The curved portion represents saturation kinetics or a zero-order reaction (excretion of a certain weight per unit time). Progressively, as the concentration of agent diminishes, the mechanisms in the renal nephron (active proximal tubular secretion) is no longer saturated, and the elimination becomes first order (k_{Tox}).

4.8.2.2. Toxicokinetics in the Lung



In the case of breathed volatiles^{3,4}, the lung is usually a relatively efficient route of elimination. Loss of the agent will frequently be observed in the blood as an initial very steep, first-order reaction (F4.20). We are looking at an initial excretion of circulating volatile chemical, plus any loosely "compartmentalized" agent (easily equilibrated back into

the blood), followed by a slow excretion of compartmentalized (adipose tissue, etc.) chemical. Examples would include such anesthetics as halothane and isoflurane, where initial post-surgical recovery is rapid, but the patient may continue to exhale low levels of the agent for hours afterward.

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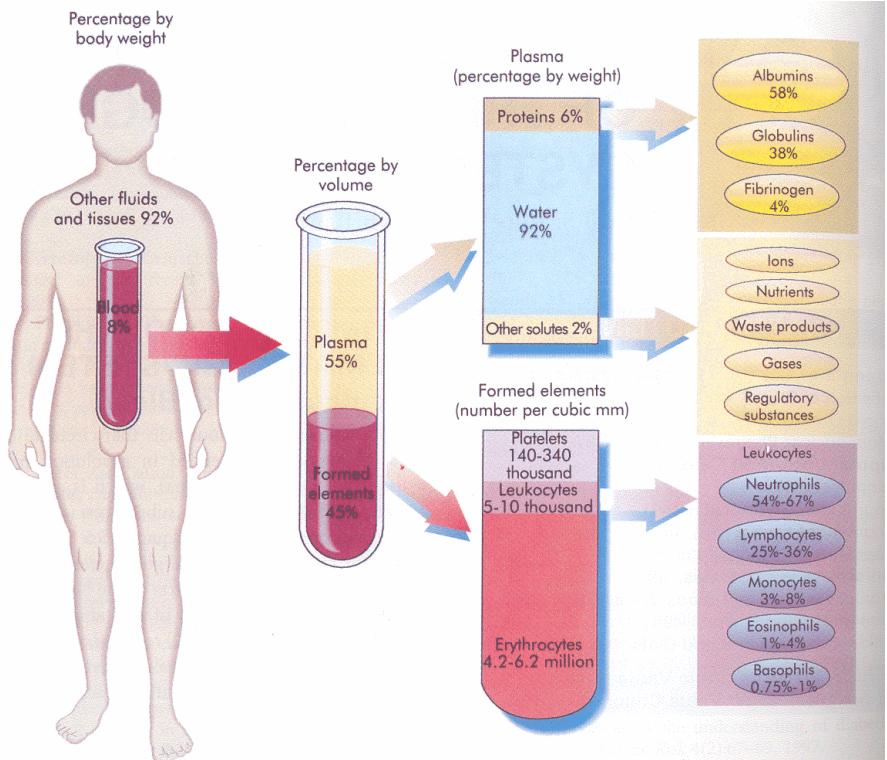
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5. Hemato- and Vascular Toxicity

5.1. Blood

F5.1. THE COMPOSITION OF BLOOD	
CONSTITUENT	MAJOR FUNCTION
WATER	SOLVENT FOR CARRYING OTHER SUBSTANCES
SALTS SODIUM, POTASSIUM, CALCIUM, MAGNESIUM, CHLORIDE, BICARBONATE	OSMOTIC BALANCE, pH BUFFERING, REGULATION OF MEMBRANE PERMEABILITY
PLASMA PROTEINS ALBUMIN FIBRINOGEN IMMUNOGLOBULINS	OSMOTIC BALANCE, pH BUFFERING CLOTTING DEFENSE (ANTIBODIES)
CELL TYPE	CELL FUNCTION
ERYTHROCYTES (RED BLOOD CELLS) 5 TO 6 MILLION PER CUBIC MILLIMETER OF BLOOD 	TRANSPORT OXYGEN AND HELP TO TRANSPORT CARBON DIOXIDE
LEUKOCYTES (WHITE BLOOD CELLS) 5,000 TO 10,000 PER CUBIC MILLIMETER OF BLOOD 	PRODUCTION OF ANTIBODIES FOR DEFENSE AGAINST INFECTION
PLATELETS 250,000 TO 400,000 PER CUBIC MILLIMETER OF BLOOD 	BLOOD CLOTTING
SUBSTANCES TRANSPORTED BY BLOOD	
NUTRIENTS (FOR EXAMPLE, GLUCOSE, FATTY ACIDS, VITAMINS), WASTE PRODUCTS OF METABOLISM, RESPIRATORY GASES (OXYGEN AND CARBON DIOXIDE), HORMONES	



Insects carry oxygen in their body using *diffusion* through internal air pipes. However, there is a critical insect size above which diffusion is not effective enough.



In crabs and some fish, the blood contains a free solution of *hemocyanin*, which binds oxygen using copper. It turns blue when oxygenated, and has only 25 % of the binding ability of mammalian hemoglobin, the molecule sequestered inside red blood cells for the purpose of binding oxygen. Horseshoe crab blood coagulates instantly when contacting *E. coli* and *Salmonella*, providing a useful diagnostic test.

There is only one vertebrate known to lack hemoglobin and red blood cells: the blackfin icefish, *Chaenocephalus aceratus*. The extremely high oxygen content of the Southern Ocean allowed it to survive the loss of globin genes, and to rely on oxygen dissolved in the plasma, a loss of 90 % in oxygen-carrying capacity. The fish has a much larger heart, blood vessels and volume of blood, and far denser capillary beds.

In mammals, an extensive capillary network supplies tissues with oxygen and removes carbon dioxide throughout the body. The difficulty of *diffusion* of oxygen and other nutrients through tissues limits the gap between adjacent capillaries to only 4 cell thicknesses.

Mammal-type circulation in an animal smaller than a shrew would be inefficient because of blood flow friction in the tiny capillaries.

5.1.1. Plasma Protein

Exogenous agents such as oxygen, once into the bloodstream, may bind more or less loosely to **plasma proteins** (F5.2), which affect distribution to the blood's cellular components and to perfused organs. Plasma protein can have a substantial role in sequestering toxicants and in slowing their release to body tissues, thereby attenuating toxic shocks.

Venoms are animal poisons evolved from saliva, that are injected and distributed in the body by the victim's circulation. *Hemotoxins* from lizards attack blood vessel walls.

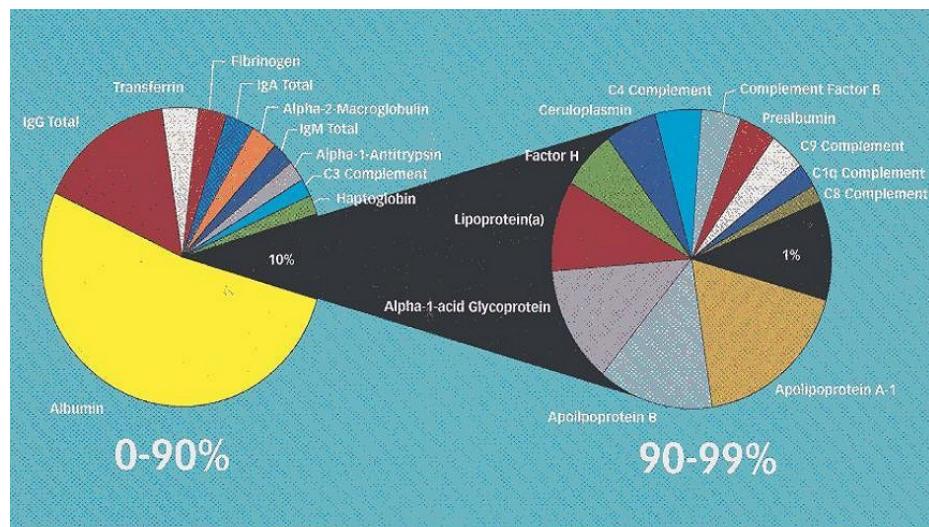
Stonustoxins from the stone fish and platypus create incredible pain. *Necrotoxins* from spiders destroy flesh. *Neurotoxins* from the black mamba paralyze victims. *Myotoxins* from rattle snakes or sea snakes attack muscle specifically. *Atraxotoxins* from funnel web spiders over-stimulate nerves. *Cardiotoxins* from jellyfish attack the heart muscle. *Batrachotoxins* used on arrow tips by some hunters induce total body system shutdown. Antibodies are useful in treatment against these bio-molecules.

Mongooses, opossums and ground squirrels produce plasma protein (protease inhibitors) that neutralize the venom of snakes found in their habitat, showing an adaptive capability of this detoxification mechanism.

5.1.1. Types of Blood Cells

The various cell types in the blood, erythrocytes, leucocytes or platelets, are differently affected by various toxicants because they are so different.

Leucocytes come in many forms, and the role of the various forms is not completely known. T lymphocytes are involved in many cellular activities that define "self" within the body (as



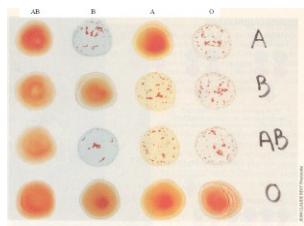
F5.2. Identification of 99 % of the protein found in plasma. Blood contains one million different protein types, with abundances that span 12 orders of magnitude.

auto-immunity), while B lymphocytes produce anti-bodies that tag foreign materials for destruction. Monocytes are immune agents dispatched by the spleen and are the first responders to trouble sites.

Even the comparatively simple red blood cells have their own complexity. For example, erythrocyte blood types define compatibility for transfusion, according to the presence of A, B and Rh⁺ antigens, but these characteristics have little to do with hemotoxicity.

F5.3. Blood transfusion incompatibilities.

Scientific American.

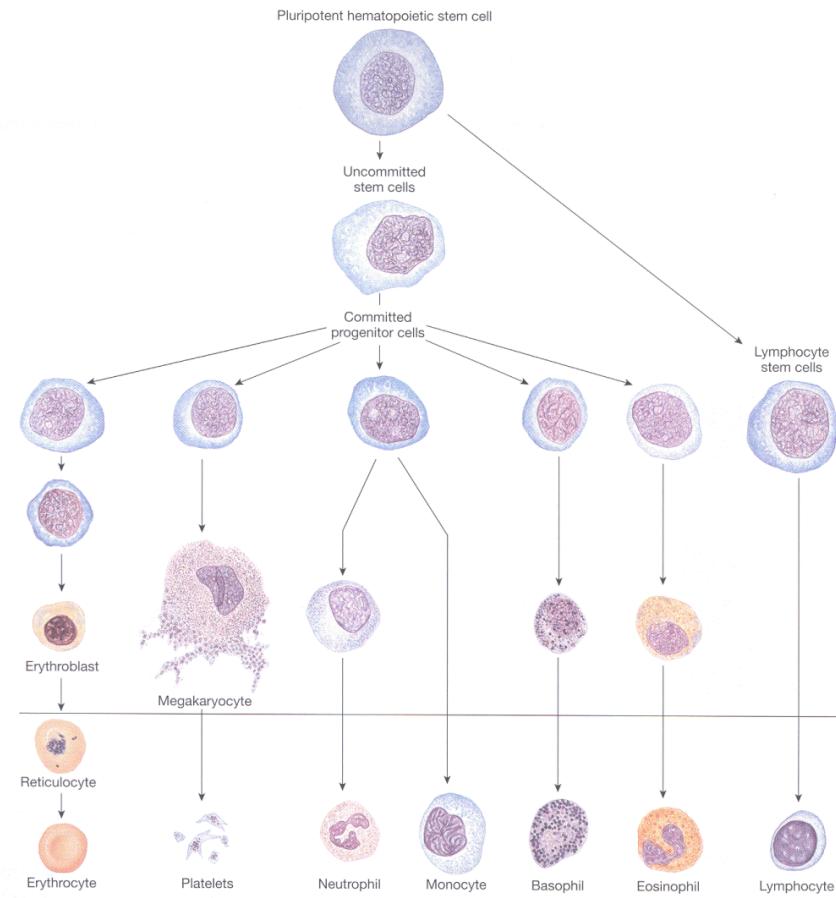


5.1.2. Formation of Blood Cells

The 5 to 6 liters of blood in the human body originate from undifferentiated mesenchymal cells or pluripotent stem cells found primarily in the bone marrow (long bones, ribs, sternum) and, in some species, in the spleen. These stem cells give rise to erythrocytes, a series of "white" blood cells or leukocytes, and thrombocytes or platelets (F5.4). Stem cell proliferation is under the influence of specific cytokines (*poietins*) arising from different tissues in response to changes in physiological status. Cytokines have a major role in stabilizing blood cell populations by stimulating the activity of cells, much like hormones do.

The difference between hormones and cytokines is circumstantial. Hormones circulate in nanomolar ($10^{-9}M$) concentrations that usually vary by less than one order of magnitude. Cytokines (ex., IL-6) circulate in picomolar ($10^{-12}M$) concentrations that can increase up to 1,000-fold during trauma or infection. Some hormones are homeostatic control systems that keep constant the concentration of hemoglobin. Others function in a transient, pulsatile fashion, triggering physiological entrainment (testosterone, cortisol, LH, FSH). Neuro-transmitters could be seen as the ultimate short-term controllers.

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F5.4. Blood cells types arising from pluripotent stem cells.

Silverthorn, 2004.

* **RH FACTOR:** antigens (agglutinogens) such as the Rh factor are present on the surface of red blood cells, leading to incompatible blood types.

An Rh- mother having her first Rh+ child is usually not a problem. But on the second child, problems start to appear because she has developed antibodies to the Rh factor, and the problems grow with each successive child. In the newborn child, hemolysis can lead to anemia and even death.

The supply of red blood cells in mammals is controlled by the cytokine erythropoietin (EPO). Hypoxic conditions, anemia, or the presence of cobalt ions will result in the release of erythropoietin from the kidney to stimulate the production of more erythrocytes. In contrast, hyperoxia or polycythemia will inhibit (or shut down) the release of erythropoietin, decreasing erythrocyte synthesis¹. In physiological situations, the concentration of EPO can change by 1000-fold. 2 million new red blood cells per second are needed to maintain cell numbers in the adult.

Any reduction in the populations of needed cells will induce an attempt by humoral regulators to restore it. There are a large number of such molecular regulators, which act on long production chains for various needed cell types.

The stem cells are at the root of the tree (F5.4). Until recently, it was assumed that many adult tissues had no stem cells and that if they had, the stem cells could only reproduce the tissue in which they resided. However, more recent evidence suggests that stem cells from the bone marrow can form nerve, muscle and other types of non-blood cells.

Implicit in this blood system, and probably in other body systems, is asymmetrical division. Stem cells given rise to two daughters, one of which replaces the mother, while the other differentiates into granddaughters.

5.1.3. Blood Tests

Blood tests are extensively used in evaluating the health status of animals and humans. Laboratory results may be outside of the *normal* range because of factors such as race, diet preferences, age, sex, menstrual cycle, degree of physical activity, improper collection and handling of the specimen, non-prescription drugs (aspirin, cold medications, vitamins, etc.), prescription drugs, alcohol intake, etc.

Labs set the normal result range for a particular test so that 95 % of healthy patients fall within. So, 5 % of healthy patients fall outside of the “norm”. Statistically, if you have 20 or 30 individual tests run as part of a panel, chances are that 1 or 2 will be slightly outside the normal range. We briefly summarize below the meaning of common blood variables for human subjects.

Glucose: High values are associated with eating before the test, and diabetes. The normal range for a *fasting* glucose is 60 -109 mg/dl. Diabetes is associated with a *fasting* plasma glucose of 126 or more. A precursor of diabetes, Impaired Fasting Glucose (IFG) is defined as readings of fasting glucose levels of 110 - 125. Sometimes a glucose tolerance test, which involves giving a sugary drink followed by several blood glucose tests, is necessary to properly sort out normal from IFG from diabetes.

Potassium is controlled very carefully by the kidneys, critical to the proper functioning of nerves and muscles, particularly the heart. Any value outside the expected range, high or low, requires medical evaluation. This is especially important if you are taking a diuretic or heart medication (Digitalis, Lanoxin, etc.).

Sodium is regulated by the kidneys and adrenal glands. There are numerous causes of high and low sodium levels, but the most common causes of low sodium are diuretic usage, diabetes drugs like chlorpropamide, and excessive water intake in patients with heart or liver disease.

CO₂ is intimately related to blood pH. Low CO₂ levels can be due to either increased acidity from uncontrolled diabetes,

kidney disease, metabolic disorders, or to chronic hyperventilation.

Blood Urea Nitrogen (BUN) is a liver waste product excreted by the kidneys. High values may signal kidney disease. BUN is also affected by high protein diets and strenuous exercise which raise levels, and by pregnancy, which lowers it.

Creatinine is a waste product largely from muscle breakdown. High values, especially with high BUN levels, indicate kidneys problems.

Uric Acid is normally excreted in urine. High values are associated with gout, arthritis, kidney problems and the use of some diuretics.

AST, ALT, SGOT, SGPT, and GGT and Alkaline Phosphatase

Phosphatase are enzymes that may be released into the blood following various forms of injury to muscles, liver and heart. Alcohol and a number of diseases cause high values.

AST/SGOT, ALT/ SGPT are liver and muscle enzymes. They may be elevated from liver problems, hepatitis, excess alcohol ingestion, muscle injury and recent heart attack.

GGT is also elevated in liver disease, particularly with obstruction of bile ducts. Unlike alkaline phosphatase, it is not elevated with bone growth or damage.

Alkaline phosphatase is an enzyme found primarily in bone and liver. Higher values are expected for growing children and pregnant women, or when damage to bones or liver has occurred, or with gallstones. Low values are probably not significant.

LDH is an enzyme present in all cells in the body. Anything which damages cells, including blood drawing itself, will raise

amounts in the blood. If blood is not processed promptly and properly, high levels may occur. If all values except LDH are within expected ranges, it is probably a processing error and does not require further evaluation.

Bilirubin is a pigment removed from the blood by the liver. Low values are of no concern. If slightly elevated above the expected ranges, but with all other enzymes (LDH, GOT, GPT, GGT) within expected values, it is probably an insignificant condition known as Gilbert's syndrome.

Creatine PhosphoKinase is an enzyme useful for diagnosis of heart and skeletal muscle diseases. This enzyme is the first to be elevated after a heart attack (3 to 4 hours). If CPK is high in the absence of heart muscle injury, this is a strong indication of skeletal muscle disease.

Albumin and Globulin measure the amount and type of protein in your blood. They are an index of overall health and nutrition. Globulin is the "antibody" protein important for fighting disease. A/G Ratio is the mathematical relationship between the above.

Cholesterol is a fat-like substance associated with heart disease. Less than 200 mg/dl is recommended.

Total Cholesterol: A high cholesterol level is a risk factor for heart and atherosclerosis. 200 mg/dl or more are too high and 240 and above are considered high risk. A low fat diet and regular exercise are recommended. There are three major kinds of cholesterol, High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL), and Very Low Density Lipoprotein (VLDL).

High LDL mark formation of deposits in the arteries. 100 mg/dl is ideal, less than 130 is recommended, greater than 160 is high risk.

High HDL is associated with low incidence of coronary heart disease.

VLDL is another carrier of fat in the blood.

Triglyceride is fat which, if elevated, has been associated with heart disease and pancreatitis, especially if over 500 mg/dl. Triglyceride levels over 150 mg/dl may be associated with other problems.

Calcium in the blood is controlled by the parathyroid glands and the kidneys. Calcium is found mostly in bone and is important for proper blood clotting, nerve, and cell activity. An elevated calcium can be due to medications such as thiazide type diuretics, inherited disorders of calcium handling in the kidneys, or excess parathyroid gland activity or vitamin D. Low calcium can be due to insufficient parathyroid hormone or drugs like Fosamax or furosemide type diuretics.

Phosphorus is also largely stored in the bone. It is regulated by the kidneys, and high levels may be due to kidney disease. When low levels are seen with high calcium levels, it suggests parathyroid disease. A low phosphorus, in combination with a high calcium, may suggest an overactive parathyroid gland.

Thyroid hormones, thyroxine (T4) and triiodothyronine (T3), are easily measurable in the blood.

Thyroxine (T4) high levels may be due to hyperthyroidism, however technical artifact occurs when estrogen levels are higher from pregnancy, birth control pills or estrogen replacement therapy. A Free T4 test (see below) can avoid this interference.

T3 Resin Uptake or Thyroid Uptake is not a thyroid test, but measures the levels of proteins that carry thyroid hormone in the bloodstream and is used to compute the free thyroxine index. A high result indicates a low level of the protein.

Free Thyroxine Index (FTI or T7) : A mathematical computation allows the lab to estimate the free thyroxine index from the T4 and T3 Uptake tests. Unlike the T4 alone, it is not affected by estrogen levels.

Free T4: This test directly measures the free T4 in the blood rather than estimating it like the FTI. It is a more reliable , but a little more expensive test. Some labs do the Free T4 routinely rather than the Total T4.

Total T3 is used when thyroid disease is evaluated. T3 is the more potent and shorter lived version of thyroid hormone. Some people with high thyroid levels secrete more T3 than T4. In these (overactive) hyperthyroid cases the T4 can be normal, the T3 high, and the TSH low.

Free T3: This test measures only the portion of thyroid hormone T3 that is "free", that is, not bound to carrier proteins.

Thyroid Stimulating Hormone (TSH): This protein hormone is secreted by the pituitary gland and regulates the thyroid gland. A high level suggests your thyroid is under-active, and a low level suggests your thyroid is overactive.

Glycohemoglobin (Hemoglobin A1 or A1c, HbA1c) : Glycohemoglobin measures the amount of glucose chemically attached to your red blood cells. Since blood cells live about 3 months, it tells us your average glucose for the last 6 - 8 weeks. A high level suggests poor diabetes control.

5.1.4. Natural Hematological Diseases

The major natural diseases of the blood are red blood cell insufficiencies (anemias) and leukocyte proliferation (leukemias).

F5.5 directly displays the hematocrit (% volume of red blood cells) for various conditions, which is normally 40-54 % for men and 37-47 % for women. Sports competitors may induce artificial polycythemia (last tube in F5.5) by re-injecting their stored blood cells before a competition.

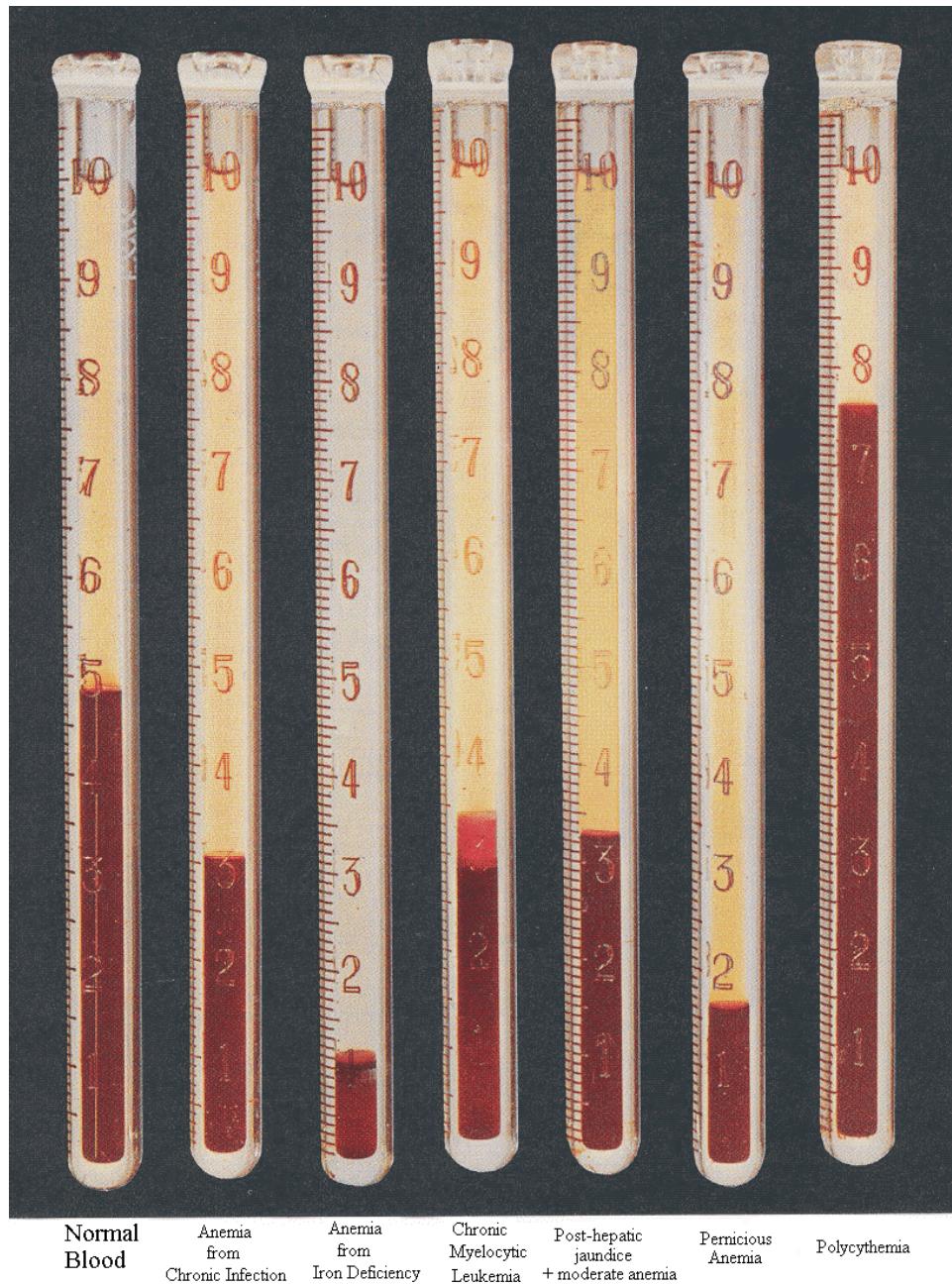
5.1.4.1. Anemia

Anemia results from insufficient delivery of oxygen to body tissues as a result of too few or defective red blood cells.

Chemical exposures can increase or decrease blood cell levels because the marrow-based process of generation of blood components is complex and delicate. Problems with red blood cells are common even without intoxication:

- Microcytic Hypochromic: too small RBCs from iron deficiency or hemorrhage. This problem can also be caused by *lead exposure*.
- Megaloblastic Microcytic: too small RBCs from folic acid or B₁₂ deficiency.
- Aplastic: too few RBCs because of a dysfunction of blood forming systems.

Red blood cell destruction or fragility can result from genetic diseases (sickle cells), malaria or chemical exposure.



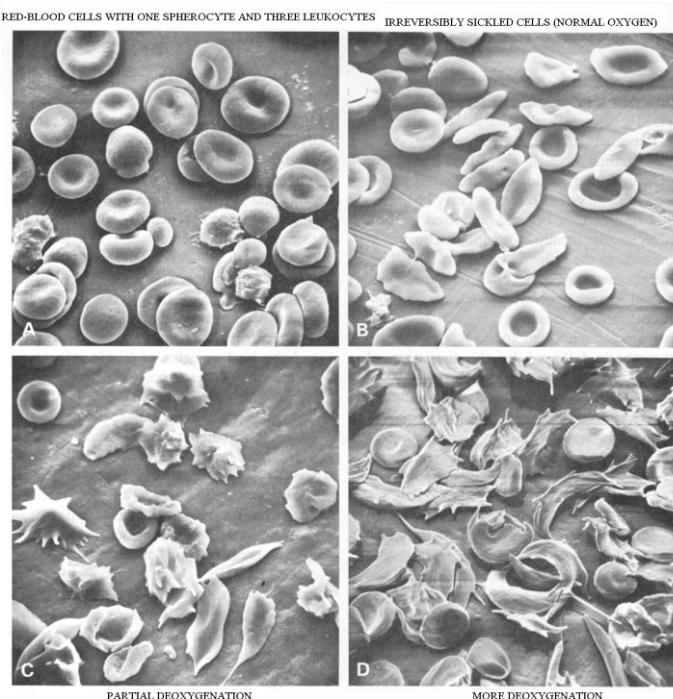
F5.5. Appearance of blood in various diseases. Wintrobe, 1974.

Chemicals can induce anemia, in part because red blood cells are exposed early to chemicals and have limited means of metabolic defense.

In mammals, they lack a nucleus and mitochondria. They produce ATP by anaerobic metabolism (glycolysis) because mitochondria would use up the oxygen that RBCs are intended to transport.

Damage to the erythrocytes may induce *hemolysis*.

Hemoglobin spilled into the plasma dissociates its four tightly bound sub-units and causes kidney toxicity by precipitating in the glomerulus. There is damage to the glomerular membrane, hematuria and a gel-like precipitation in the tubular lumen, thus impairing renal function. It also interacts toxicologically with a number of other molecules throughout the body.



F5.6. Sickle cell transformation. Wintrobe, 1974.

Destruction of red blood cells also impairs oxygen and carbon dioxide transport. The result is hypoxemia (insufficient supply of oxygen to tissues) and secondary toxicity in tissues which require large supplies of oxygen (CNS, heart).

5.1.4.2. Sickle Cell Anemia

Sickle cell anemia is a relatively common genetic condition, that amplifies problems related to oxygen insufficiency. It is thought to result from an adaptation giving immunity against malaria. The sickle mutation increases a protein, heme oxygenase-1, which increases CO concentrations. The binding of CO to heme reduces the toxicity that occurs when heme is released into the blood, as erythrocytes disintegrate²⁵.

The sickle cell transition is due to the spontaneous crystallization of the variant HbS, triggered by low oxygen atmospheres.

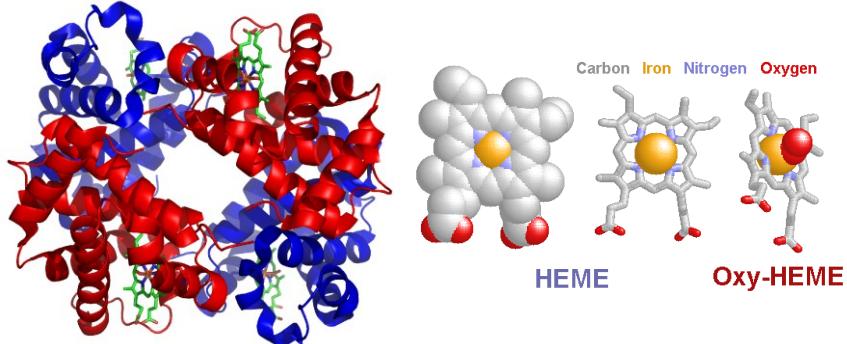
It appears that specific molecules on the deformed cells make them stickier than healthy red blood cells, contributing to painful sickle crises. Sickle cells are normally eliminated by the spleen. The condition is most prevalent in black women. Individuals that are homozygous for HbS are not usually in the workforce. Individuals that are heterozygous show almost no impairment in most situations. It may be appropriate to screen in special cases such as operation of aircraft and submarines. Genetic trials in rats aimed at correcting sickle cell disease have been successful¹².

5.2. Hematotoxicity

5.2.1. Oxygen Transport

The erythrocyte has a simpler metabolism than normal cells. There is no glycogen in the erythrocyte, therefore, glucose

must be available from the blood. It is metabolized by two routes: the Embden-Meyerhof pathway (anaerobic) and the pentose phosphate pathway (aerobic). Routine detoxification of free radicals in the erythrocyte is heavily dependent on glutathione (page 3-19).



F5.7. Views of hemoglobin. Left, the complete molecule where the protein subunits are in red and blue, and the iron-containing heme groups in green. Right, the oxygen binding site (heme).

The erythrocyte carries **carbon dioxide** away from tissues. A small amount is carried in free solution, 75 % is transported as bicarbonate ion (HCO_3^-) converted by the enzyme carbonic anhydrase (a soluble enzyme in the intracellular fluid), the balance of the carbon dioxide is combined with free amino groups in the globin to form carbamino ($\text{R}-\text{NH}-\text{COOH}$).

30 % of the erythrocyte is made of the protein **hemoglobin**. **Hemoglobin** (Hb) is responsible for the transport of oxygen, attaching it to a ferrous (Fe^{2+}) ion which is held in position via chelating bonds to porphyrin ring N-groups. One free

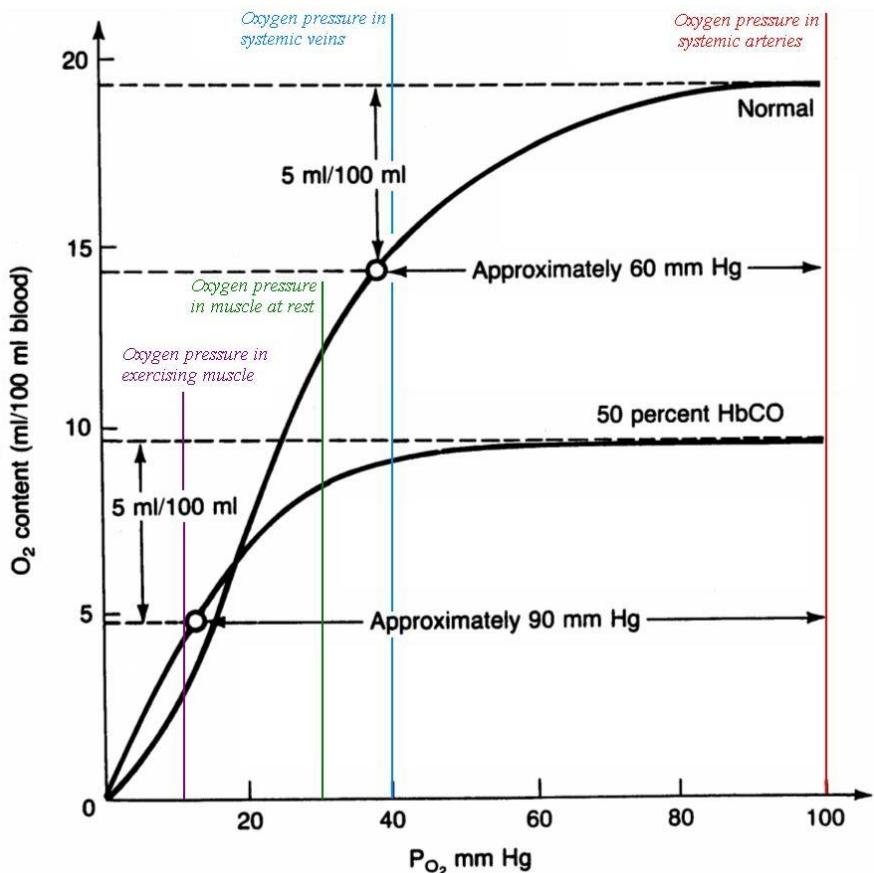
attachment of the Fe^{2+} is an imidazole group of the protein globin, which leaves one bond free for the oxygen (F5.7). **Heme** is a porphyrin chelate of iron in which the iron is Fe^{2+} , the oxygen-carrying, color-furnishing, non-protein part of Hb. In the disease called porphyria, mutated enzymes manufacture defective heme, which builds up to toxic levels.

The performance of Hb as an oxygen carrier is described by the oxygen saturation curve of blood. The curve defines the volume of oxygen released under various partial pressures of gaseous oxygen (F5.8). In order to prevent hypoxia in distal tissues, Hb loses its affinity for oxygen when the oxygen pressure is low.

Hemoglobin was originally a detoxification molecule used by simple animals as a defense against the oxygen "pollution" from plants. For example, the hemoglobin of *Ascaris lumbricoides* (a worm living in low oxygen environments) binds oxygen 25,000 more tightly than human Hb. Myoglobin in muscle has an even stronger affinity for oxygen than hemoglobin, making oxygen delivery to muscle tissues possible.

It has been demonstrated experimentally that there are alternative fluids to hemoglobin that can carry enough dissolved oxygen to sustain life (F5.9).

Without oxygen, animal metabolic pathways end up with excessive lactic acid. Turtles can cope with large amounts of lactic acid because they neutralize it using the calcium carbonate in their shell. Carps and goldfish can convert lactic acid to ethanol, which can be released from their gills¹⁵.



F5.8. Saturation curve of blood with air and CO.
Atmospheric air has 160 mm Hg of O₂.
Modified from Williams and Burson, 1985.

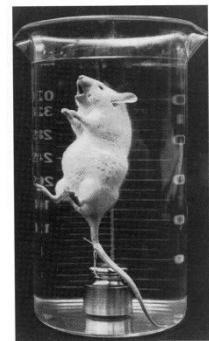
5.2.1.1. CO Interference with Oxygen Transport

Carbon monoxide binds Hb 225 times more strongly than O₂, as shown in the equation below, where M = 225 and the Ps represent partial pressures. In the presence of CO, therefore, less Hb is available to carry oxygen.

$$\frac{Hb - CO}{Hb - O_2} = M \frac{P_{CO}}{P_{O_2}}$$

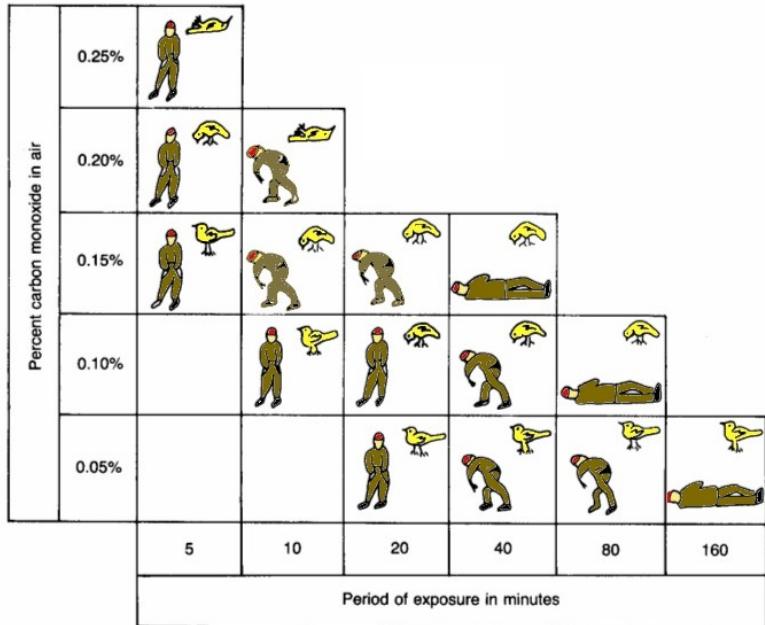
F5.9. A rat breathing perfluorocarbons.

0.07 % CO can be lethal. Another effect of CO at 0.1 % is to alter the Dissociation-Saturation curve of Hb, as shown in F5.8. Saturation of Hb with CO inhibits oxygen transport by binding less oxygen more tightly: in the graph, a depression of 90 mm Hg in oxygen pressure (as opposed to 60 mm) is necessary to release 5 ml of oxygen. The symptoms of CO poisoning are low blood pressure, fainting, dizziness, headache, weakness and nausea.



In a Toxicokinetic application, **canaries** have been used as warning devices to protect the health of miners, since coal mines are likely to contain pockets of CO. A canary is small, its metabolism is high, and it has a high breathing rate. Therefore, it will be in equilibrium with CO much faster than a human, and should serve as a warning for humans to leave the tunnel. However, the binding of CO to canary Hb is only 110 times (not 225) stronger than that of oxygen. Because of this, it is possible that the miner will die before the canary, usefully warning the canary to get out of the mine shaft. Therefore canaries as a warning device are only effective above 0.2 % CO.

Because the CO₂ chemo-receptors that increase respiration rate are not affected by CO poisoning, the lack of O₂ concentration into the tissues is not noticed until too late.



F5.10. Effects of Carbon Monoxide on Man and Canaries.
Ann. Occup. Hyg. 5: 1961.

Carboxyhemoglobin (CO-Hb)

- Individuals with previous coronary artery disease may develop angina with moderate activity at levels as low as 3-5 %. Cigarette smokers are chronically around 5 %. Exposure of 35 ppm CO for 10 hours is equivalent to 5 %.
- Cardiac compensatory effects seen at 8-10 %: heart attack, lightheadedness, chest pain.
- Electrocardiogram problems (extrasystoles, premature ventricular contractions, fibrillation) at 10-25 %.

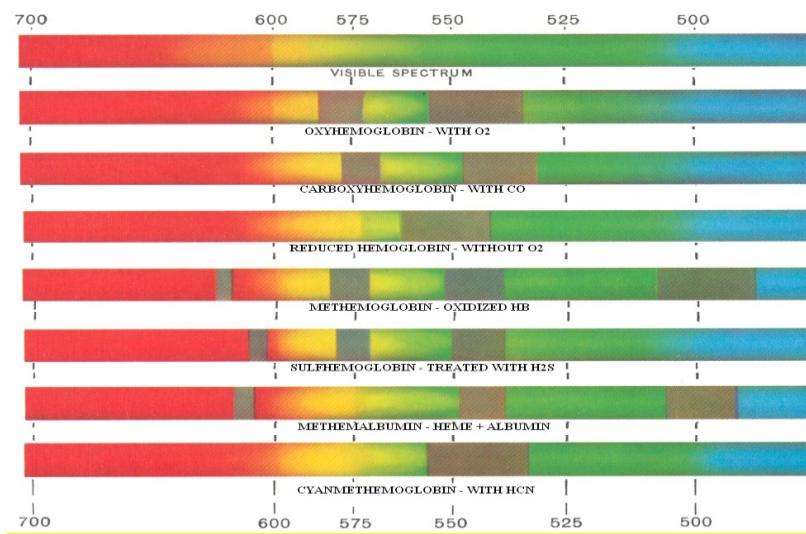
Treatment: Carboxyhemoglobin pressure can be lowered by breathing oxygen through a mask for a few hours. It also combats nausea, headache and loss of consciousness commonly experienced by victims. However, hyperbaric

oxygen should be the treatment of choice for seriously poisoned people to prevent memory lapses, confusion and language problems.

In the weeks following their poisoning by CO, some survivors develop concentration problems, personality changes or sensory impairments. These effects can be traced to misguided immune responses to a brain protein, myelin basic protein, that is altered by CO gas¹⁴.

CO binds mitochondrial enzymes and myoglobin, increases platelet stickiness and decreases arrhythmia thresholds.

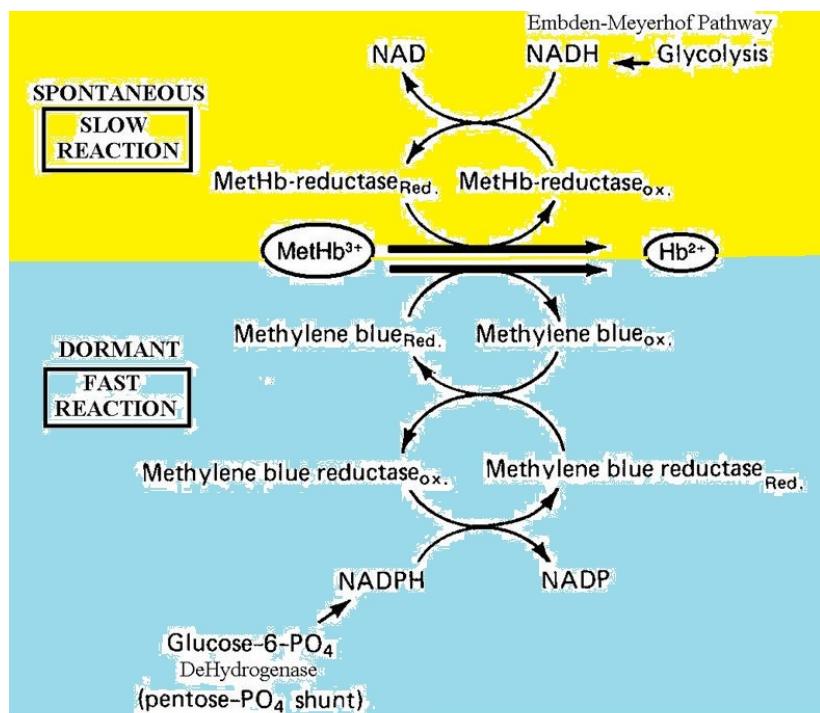
Methylene Chloride is a solvent used in degreasing and paint stripping which is absorbed by respiration or through the skin, and is metabolized in the blood to CO. It may elevate COHb to 10 % or more in poorly ventilated spaces. It is also a carcinogen.



F5.11. Spectra of Hemoglobin. Modified from Wintrobe, 1974.

5.2.1.2. Chemical Attacks on Hb

Unfortunately, Hb is not immune to chemical attacks, as revealed by the dramatic changes in the color of hemoglobin (F5.11) in the presence of oxygen (ruby-red), carbon dioxide (blue-red), carbon monoxide (cherry red), and cyanide (bright red). The erythrocyte has limited ability to detoxify activated oxygen which, if present in excess, becomes toxic to the cell. *Hemoglobin can be pathologically oxidized to MetHemoglobin.*



G-6-PD in red blood cells converts methemoglobin back to hemoglobin. Humans genetically deficient in this enzyme can have 30% methemoglobin in their red blood cells. Treatment with reducing agents like ascorbic acid (Vitamin C) or methylene blue considerably lowers the amount of methemoglobin in their blood.

The main clinical manifestation of this deficiency is (induced) haemolysis. This is usually the result of the existence of an oxidizing factor from the environment: a drug, an infection, some foods, chemical exposure. In most cases this passes unnoticed because the organism is able to counteract. Most patients live a perfectly normal life without ever noticing the effect of this disorder.

METHYLENE BLUE

Methylene blue is an artificial dye first used as a marker in studying bacteria and body tissues. Methylene blue, which can be taken by mouth or injected intravenously, imparts a blue color to urine and feces. It is also an antiseptic (disinfectant), although its bactericidal properties are very mild, and a mild urinary antiseptic and stimulant to mucous surfaces. It has been used as an antiseptic in urinary tract infections and as an indicator dye to detect certain compounds present in urine. Methylene Blue has also been shown to minimize the occurrence of kidney stones, and may prevent their formation.

Its present uses are for its oxidation-reduction functions (methemoglobin regeneration to hemoglobin), tissue staining, and as an anti-aging drug. Methylene Blue activates a normally dormant reductase enzyme system which reduces the Methylene Blue to leucomethylene blue, which in turn is able to reduce methemoglobin to hemoglobin. It is believed to be reduced in the tissues to the leuco form, which is slowly excreted mainly in the urine, together with some unchanged drug. In large doses, Methylene Blue can produce methemoglobinemia.

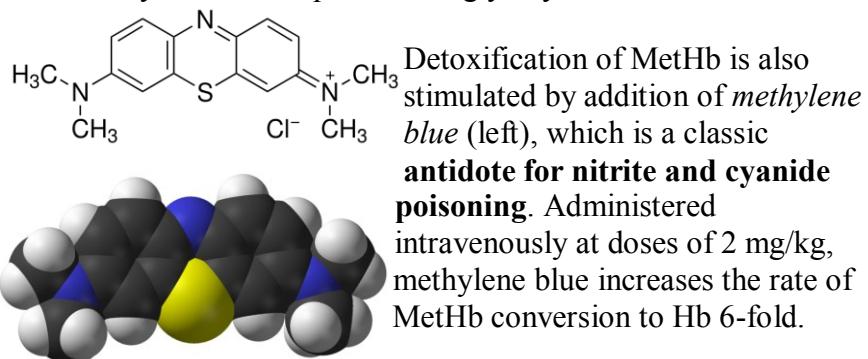
The iron in the Heme of Hb is transformed to the ferric state (Fe^{+++} , an “electron” is lost) rather than the useful ferrous state (Fe^{++}). MethHb is greenish brown to black in color and **does not bind oxygen or CO**; it binds water instead. Normal level in the blood are 1-2 %, it becomes dangerous at 15 %. Hb in erythrocytes can be reduced back to the ferrous state by two metabolic routes (F5.12):

- a **spontaneous** system of detoxification based on reduced nicotinamide adenine dinucleotide (NADH) generated by the glycolytic Embden-Meyerhof pathway. Cytochrome b_5 and cytochrome b_5 reductase are the enzymes responsible for changing MetHb back to Hb by maintaining NADH supply. 95 % of MetHb is processed this way, and

F5.12. Reactivation of methemoglobin (MetHb) to hemoglobin (Hb) following chemical reduction.
Methylene blue can be used to reactivate Hb in emergencies.

- a **dormant** system based on glucose-6-phosphate-dehydrogenase (G-6-PD), involving methemoglobin reductase, otherwise known as the “glucose-monophosphate shunt” and generating the co-factor reduced nicotinamide-adenine dinucleotide phosphate (NADPH). This accounts for 5 % of the regeneration activity.

Both systems are dependent on glycolysis.



Detoxification of MetHb is also stimulated by addition of *methylene blue* (left), which is a classic **antidote for nitrite and cyanide poisoning**. Administered intravenously at doses of 2 mg/kg, methylene blue increases the rate of MetHb conversion to Hb 6-fold.

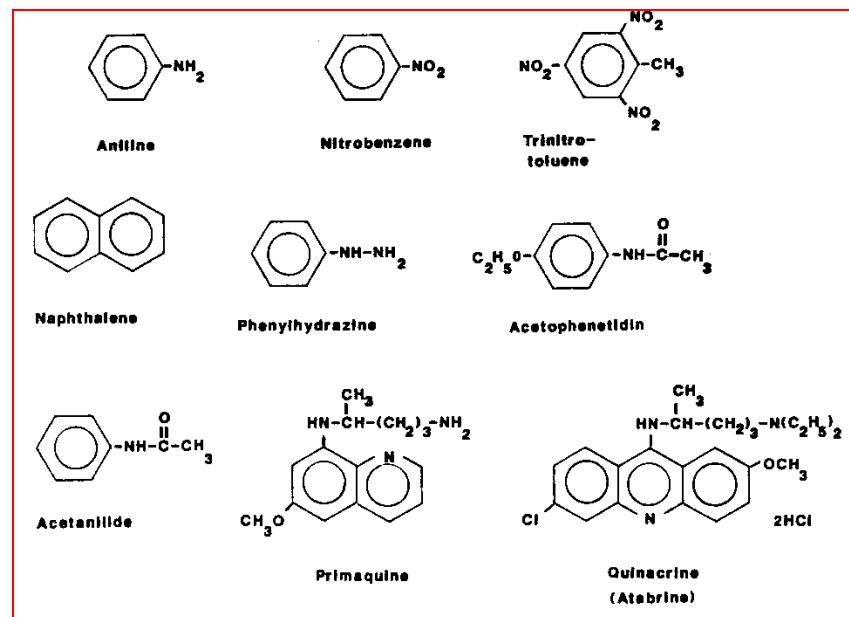
The vast majority of work-related hemolytic anemias are induced by exposure to benzene derivatives or aromatic ring structures. These agents are potential oxidation-reduction catalysts, acting as oxygen scavengers, water soluble enough to be carried in an aqueous medium but lipid soluble enough to penetrate the erythrocytic membrane, gaining access to a vast storehouse of oxygen. In the ensuing reaction, both the chemical and the oxygen become free radicals.

MetHb generating chemicals (oxidizers):

- nitrites (-NO₂),
- nitrates (-NO₃, need to be activated to nitrites in the gut), aromatic amines (NH₂-Benzene),

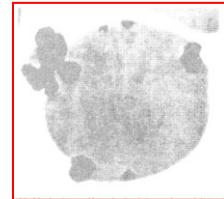
- chlorate salts (Na- O-O-O-Cl).

These chemicals can produce hypoxia in the exposed. See also figure 5.13.



F5.13. Structures of chemicals causing hemolysis or methemoglobinemia.

Hb can also be *irreversibly* denatured or oxidized by chemical agents. In this case, *Heinz bodies* are observed that are covalently bound to the inside of the Red Blood Cell membrane (F5.14). These cells are not toxic by themselves, but will usually hemolyze because of loss of flexibility, which can lead to anemia.



F5.14. Heinz bodies in an erythrocyte.
 x 26,000. Rifkind, 1965.

5.2.1.3. Exposure Examples

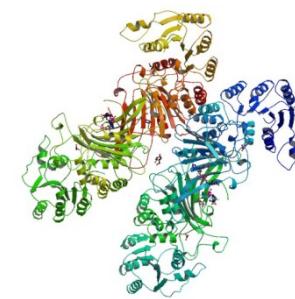
Chemical-induced hemolysis began in the 1800's German dye industry when coal tars were converted into basic industrial chemicals, including anilines, nitrobenzenes, quinones and naphthalene.

Hemolytic anemias can be caused by such metals as lead, mercury, copper, as well as arsenic (as arsine), all of these cytolytic metals are highly reactive with sulphhydryl groups (-SH) that are abundant in erythrocytes in the form of glutathione, hemoglobin, etc. Cases...

- 1) Explosives workers, called "blue workers", due to chronic cyanosis resulting from methemoglobin formation as a consequence of exposure to nitro-benzenes and nitrotoluenes.
- 2) Antimalarial drugs related to 8-amino-quinoline such as pamaquine and primaquine, used in WW II in the Pacific theatre, resulted in massive hemolytic crises among black soldiers. It was recognized that there was a susceptible subgroup within this population.
- 3) The analgesics acetanilide and acetophenetidin have been removed from the over-the-counter market because of patient hemotoxicity, particularly with abuse.
- 4) A defoaming agent in beer, an aniline-related chemical, caused "blue workers" in a Montreal brewery exposed to leaking defoamer³⁻⁷.
- 5) Repeated development of new drugs based on phenylhydrazine.

5.2.1.4. The Genetics of Erythrocytic Hemolysis

In most individuals, the mechanisms of detoxification described previously are sufficient to deal with any MetHb and oxygen radical formation. However, the most common enzyme deficiency in humans is caused by a variety of mutations to band Xq28 of the "X" chromosome. Severe reductions of the enzyme glucose-6-phosphate dehydrogenase (G6PD, shown), impairs the "rapid" protective glucose monophosphate shunt system, leaving individuals unable to cope with oxygen stress. These subjects are particularly sensitive to red cell hemolysis induced by chemicals, because *glutathione* cannot be regenerated. The deficiency is rare among Caucasians but, among blacks, some 12-14 % of males, but only 2 % of females have the deficiency.



Humans genetically deficient in G6PD can have 30 % MetHb in their red blood cells. Treatment with reducing agents like ascorbic acid (Vitamin C) or Methylene Blue considerably lowers the amount of MetHb in their blood. The main clinical manifestation of this deficiency is (induced) hemolysis. In most cases this passes unnoticed, because the organism is able to compensate. Most patients live a perfectly normal life without appreciable effects of this disorder.

When it becomes noticeable, it is usually the result of an oxidizing factor from the environment: a drug, an infection, some food or chemical exposure.

The key cellular oxidant scavenger in this pathway is the tripeptide, reduced glutathione (GSH, gamma-L-glutamyl-L-cysteinyl-glycine), formed by the hexose-pentose monophosphate shunt. Relatively high levels (3-5 mM) of the reduced form are normally present in erythrocytes, with only minuscule amounts of the oxidized (GSSG) dimer.

Glutathione is a radical scavenger that works rapidly (with or without the enzyme glutathione-s-transferase, a Phase II conjugating enzyme) to neutralize highly reactive intermediates capable of damaging and weakening the erythrocytic membrane. The inability to maintain adequate levels of GSH destabilizes the cellular protective mechanism, resulting in weakened and easily ruptured erythrocytic membrane and enhancing hemolysis.

The syndrome leads, upon exposure, to methemoglobinuria, formation of sulfhemoglobin (only partially soluble in erythrocytes) which precipitates into spheroidal bodies (Heinz or inclusion bodies), alteration of the shape, rigidity, and viability of the red cell, causing hemolysis and resulting in massive hematuria.

It is important to point out that Heinz body hemolytic anemia can occur in normal (not enzyme-deficient) individuals, but only if exposure to a chemical oxidant is several fold higher than that producing hemolysis in the G-6-PD deficient person.

5.2.2. Metabolic Anoxia: Cyanide and H₂S

Hydrogen cyanide is a colorless gas or liquid. About 30 % of people are unable to detect cyanide gas's (HCN) bitter almond odor, but those who can often smell cyanide at autopsy of a poisoning victim. The lethal dose of sodium cyanide by mouth is 100 mg, and 2000 mg/minute x m³ when inhaled. Effects are confusion, dizziness, increased breathing and heart rate,

Chemical-induced methemoglobinemia case history.

Levy and Wegman, Occupational Health, Little and Brown, 359-362, Boston, 1983.

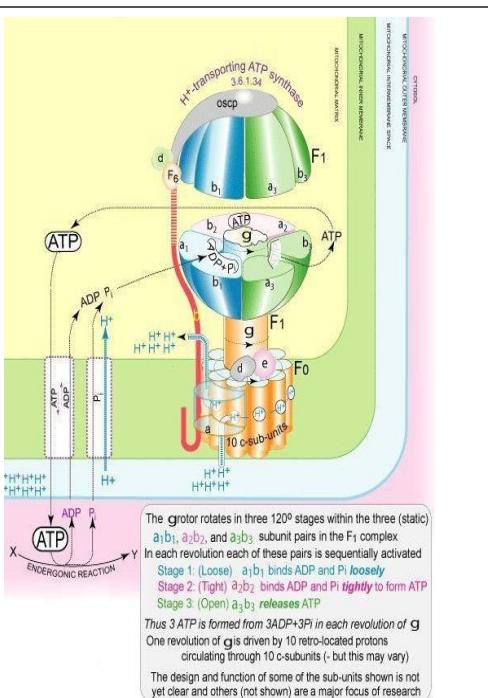
The patient was a well-built, vigorous, 50-year old man who had been employed for 21 years as a laborer in a dyestuff plant where he was in daily contact with aniline. Among his regular duties was the refilling each morning of a barrel containing industrial grade aniline. The barrel, in which aniline was stored under pressure, was located on the floor of a room shared by three employees. Aniline from this sealed barrel was pumped as needed via tubing into the dye-application work area by means of hydraulic pressure.

Beginning about two years prior to the accident, the man complained to his wife of a gradually worsening, unremitting headache. For two months she in turn had noted bluish discoloration of her husband's lips, ears, and fingertips, most evident on his return home from work each evening. One midsummer morning, just after the patient had filled the reservoir barrel with a fresh supply of aniline under sealed pressure, the bottom of the barrel blew out. Roughly 30 gallons of aniline gushed forth, flowing over the patient's clothing and inundating the floor of the work area. He, as well as the two co-workers standing nearby, rapidly experienced nausea, dizziness, and faintness, but after cleaning up the spilled chemical all resumed their work until noon. Two hours later the patient returned to work but grew dizzy, nauseated, and began to vomit. (The two co-workers had called in to report themselves too ill to return to the factory.) The patient's physician was called, and immediate hospitalization was arranged.

On admission, the patient was described as confused, agitated, breathing rapidly and deeply, and displaying dark cyanosis of the hands, feet, and ears. His mucous membranes also appeared grey-blue, particularly when viewed under light from a quartz-mercury lamp. Freshly drawn blood appeared dark maroon-brown. There was no anemia, but the reticulocyte count was 5.8 percent and, after supravital staining, 6.8 percent of his red cells displayed Heinz bodies. About 60 percent of the patient's hemoglobin was in the form of methemoglobin (ferrihemoglobin). Within less than 24 hours the hematocrit dropped moderately, the reticulocyte level rose (and continued to rise for several days), and the portion of red cells containing Heinz bodies increased to nearly 30 percent. The methemoglobin concentration fell abruptly during the initial 15 hours and by the second day only traces remained. However, 15 percent of the hemoglobin at that time was in the denatured form of sulfhemoglobin. By day 4, despite several transfusions, the anemia had become more severe, the reticulocyte count was 11 percent and the level of Heinz-body-containing cells was 28.8 percent. Nevertheless, the patient felt much improved and complained no longer of dizziness or nausea. Urine specimens on admission and during the several days thereafter contained high concentrations of the oxidant hydroxyl derivative of aniline, p-aminophenol.

In the ensuing week the patient gradually recovered completely from his symptoms, and the only laboratory aberrations that persisted beyond ten days were elevated levels of reticulocytes and of cells containing Heinz bodies; traces of sulfhemoglobin were detectable for several weeks. All three abnormalities cleared entirely within two months of the accident.

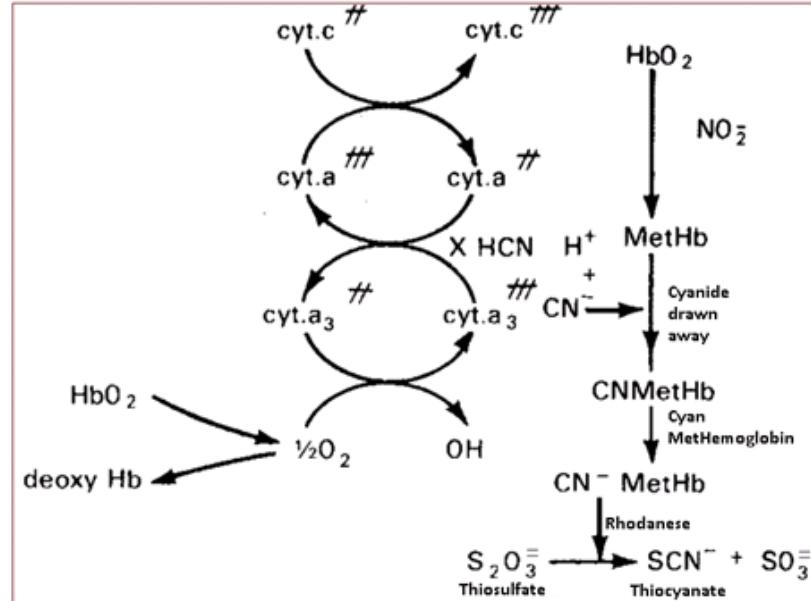
convulsions and asphyxia. In HCN poisoning, blood delivery of oxygen is normal, but HCN impedes the use of oxygen by cells.



F5.15. Synthesis of ATP within mitochondria.
Donald Nicholson, 2002.

Exposed subjects have a red color, similar to CO poisoning, since their venous blood returns still loaded with unused oxygen. Ultimately, the brain cells controlling respiration die. Cyanide specifically inhibits (see F5.15) **cytochrome oxidase a₃**, the cytochrome that normally releases ATP. The therapy for cyanide poisoning is illustrated in F5.16.

Oddly enough, the therapy involves the formation of a toxic product, MetHb. This is done by administration of inhaled amyl nitrite and by injections of sodium nitrite. The production of MetHb allows drawing CN away from cytochromes because ferric heme groups avidly bind ionic cyanide, forming cyanmethemoglobin. This is followed by the administration of thiosulfate ($\text{S}_2\text{O}_3^{2-}$). Thiosulfate provides a substrate for the enzyme rhodanese which transforms cyanide bound to Hb to thiocyanate. The thiocyanate, much less toxic, is excreted.



F5.16. Therapeutic management of cyanide poisoning.
Casarett & Doull, 1995.

Zyklon B (shown), used in concentration camps and as an insecticide, is a crystallized form of hydrogen cyanide. Most resuscitations from cyanide poisoning occur in individuals who ingested soluble salts, where absorption is delayed.

H₂S, the gas with a rotten egg odor, is another chemical which impairs the use of oxygen by body cells (cytotoxic hypoxia). 80 ppm of hydrogen sulfide can reversibly bring the metabolism of mice to a standstill, lowering their body temperature from 37° to 11° C. The animals reduced their use of oxygen by a factor of 10, lowering their heart and breathing rates²⁰. This characteristic might be used to attenuate ischemic damage during emergency situations.



C. elegans, when grown in 50 ppm H₂S, the OSHA human exposure limit, lives 70 % longer than worms living in normal air¹⁹.

H₂S is naturally produced in the body, and manages many important biological functions, in particular blood pressure²¹. Few poisons are more rapidly acting than inhaled hydrogen sulfide, which is common to sewers and oil fields.

The treatment is similar to that of CN poisoning, but there are rarely trained individuals available, and prepared with an intravenous nitrite injection.

5.2.3. Alterations in Cellular Composition

Many chemicals can attack the blood, resulting in obvious changes in blood cellular composition. In most cases, the exact mechanism of toxicity is unknown. Toxicity to stem cells, which largely control the ultimate composition of the downstream differentiated cells, needs to be defined in a special way, different from toxicity to differentiated (somatic) cells, particularly in their relation to oxygen.

Differential blood counts can document imbalances of blood composition resulting from exposure to toxicants.

5.2.3.1. White Blood Cell Imbalances

Exposure to some chemicals (benzene) can induce leukemia. Chemicals that reduce white blood cell counts (leukopenia) lower resistance to infection (DDT decreases monocytes and lymphocytes).

5.2.3.2. Thrombocytopenia

Low platelet counts produced by certain chemicals may result in blood loss. There are other mechanism for blood loss. For example, warfarin inhibits fibrin formation from fibrinogen, while aspirin inhibits platelet aggregation.

5.2.3.3. Chemicals causing Thrombocytopenia

Acetaminophen (Tylenol), Aminopyrine, Aspirin and salicylates, Benzene, Bismuth, Chloramphenicol, Chlordane, Corticosteroids, Dextropropoxyphene (Darvon), Diazepam (Valium), Diethylstilbestrol, Digitoxin, Dimercaprol, Disulfiram (pesticide), Insulin, Isoniazid, Lindane^Ω (pesticide), Mercurials, Phenobarbital, Phenylbutazone, Potassium Iodide, Quinidine, Quinine, Stilbestrol, Tetracycline, Toluene diisiocyanate (manufacture of elastomers), Trinitrotoluene (TNT).

5.2.3.4. Chemicals causing Hemolysis

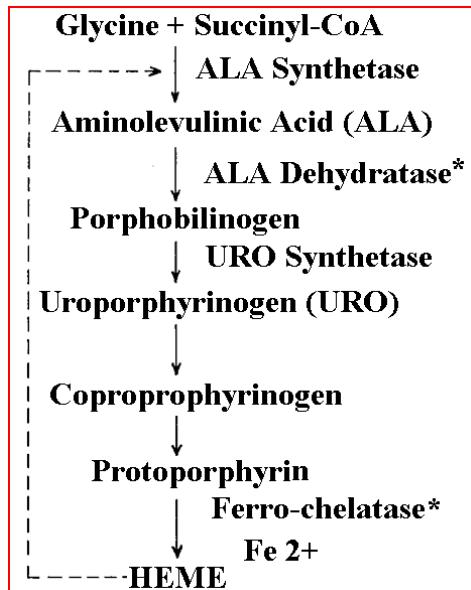
Arsine, Benzene, Butyl Cello solve (dry cleaning), Carbutamide, Chloramphenicol, Chlorpromazine, Dimercaprol, Lead, Mephentyoin, Methyl chloride, Naphthalene, Nitrobenzene, Phenylbutazone, Phenylhydrazine (dye synthesis), Primaquine, Quinacrine, Streptomycin, Tolbutamide, Trinitrotoluene.

5.2.3.5. Chemicals causing Anemia-Pancytopenia

Alkylating agents, Amitriptyline, Ampicillin, Arsenicals, Arsphenamine, Aspirin, Benzene, Carbon Tetrachloride, Chloramphenicol, Chlordane, Chloroquine, Colchicine, Diazepam, Gold compounds, Hydrochloroquine, Insecticides, Isoniazid, Lindane, Meprobamate, Methimazole, Oxyphenbutazone, Phenylbutazone, Potassium Perchlorate (explosive), Propylthiouracil, Quinacrine, Quinidine, Salycilates, Streptomycin, Sulfa drugs, Tetracycline, Trinitrotoluene.

^Ω

Canada has banned lindane.



F5.17. Synthesis of Hb and mechanism of action of Pb, directly (*) and indirectly by feedback.

5.2.3.6. Example: Lead Poisoning

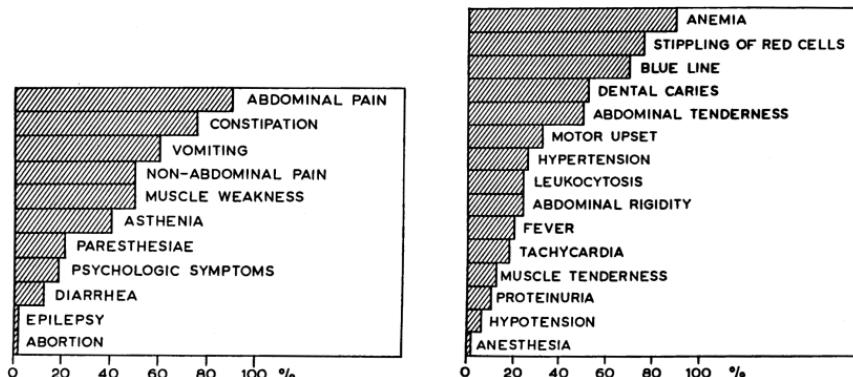
Lead intoxication is a on-going problem in numerous industries (smelters, brass foundries, painters, miners, garage workers, paint removal by blowtorch or power sander). Hypertension, reproductive problems, decreased kidney and brain function are common. Hematologic symptoms are generally chronic in nature, and marked by the insidious development of:

- (1) chronic hemolysis,
- (2) microcytic, hypochromic anemia, and
- (3) sideroblastic (iron-related) changes in bone marrow.

Lead translocates to the long bones where erythropoiesis occurs, interfering with the synthesis of the heme (porphyrin) precursor to hemoglobin (F5.17), by inhibiting the key enzyme ferrochetalase, the last step in heme synthesis. In this scheme, the initial step in the heme pathway is controlled by a feedback

mechanism on the enzyme aminolevulinic acid synthetase (ALA-S) based on the body's need for heme. With sufficient heme being manufactured, ALA-S does not function, whereas with the blocked ferrochetalase, there is no heme formed and ALA-S continues to make aminolevulinic acid, which is used by other enzymes to make porphyrin intermediates. Levels of these intermediates build up in the body, urinary excretion of ALA is elevated (one diagnostic test) as is excretion of a number of the protoporphyrins (both fecal and urinary excretion are diagnostic).

The symptoms and signs of intoxication are shown in F5.18. One specific sign of plumbism is the lead "line", a deposit of blue-black lead sulfide deposited into the gums immediately above the teeth (F5.19).



F5.18. Symptoms (left) and signs (right) in lead poisoning. Dagg et al.

Typical hematologic changes of lead poisoning in adults (F5.20) include:

- 1) hypochromic, microcytic erythrocytes,
- 2) basophilic stippling in erythrocytes: "granules" of semi-precipitated protein,
- 3) presence of siderotic granules (containing iron),

- 4) reticulocytosis - reticulocytes being released into blood,
 5) increased osmotic fragility of erythrocytes, erythroid hyperplasia in bone marrow which causes #1 above.

F5.19. Lead lines. Belknap, Wintrobe, 1974.

	Normal	Lead Poisoning
Hemoglobin (g/dl)	♂14-18 ♀12-16	10.7 (8-13)
Volume Packed Red Blood Cells (l/l)	♂ 0.40-0.54 ♀ 0.37-0.47	0.35 (0.29-0.43)
Mean Cell Volume (fl)	89 (83-96)	79 (70-92)
Mean Cell Hb Content (g/dl)	34 (32-36)	31 (27-36)
Reticulocytes (%)	1.6 (0.6-2.7)	4.4 (1.5-11.6)
Stippled cells (%)	rare	1.8 (0.1-7.5)
Leukocytes ($\times 10^9/l$)	4-11	4-15



F5.20. Hematologic changes in the anemia of lead poisoning in adults.

Wintrobe, 1974.

If you have been intoxicated with lead, what can you do? EDTA can be used to chelate lead out of the body. For milder forms of intoxication, it appears that supplements of 1.2 g per day calcium can reduce blood levels of lead by 31 %²³.

5.2.3.7. Example: Benzene Poisoning

A study of hematotoxicity among Chinese workers heavily exposed to benzene (Rothman et al.) produced the results of F5.22: a decrease in white blood cells, absolute lymphocyte count and platelets.

Although drugs (chloramphenicol, sulphonamides, acetazolamide, phenylbutazone, mephenytoin, hexachlorocyclohexane) and anti-cancer agents (methotrexate, alkylating agents such as BCNU) can induce aplastic anemia (failure of bone marrow stem cells to produce an adequate number of erythrocytes, granulocytes, and platelets), benzene accounts for 5 to 10 cases of fatal aplastic anemia per year.

	Absolute lymphocyte count		RBC	Hematocrit	MCV	Platelets
	WBC Mean ± sd (range)	Mean ± sd (range)	Mean ± sd (range)	Mean ± sd (range)	Mean ± sd (range)	Mean ± sd (range)
Control (n = 44)	6.8 ± 1.7 (3.1-12.5)	1.9 ± 0.4 (1.1-2.8)	4.7 ± 0.6 (3.0-5.6)	42.0 ± 5.6 (27.7-50.7)	88.9 ± 4.9 (71.2-96.6)	166 ± 59 (62-313)
Exposed						
≤31 ppm (n = 22)	6.4 ± 1.8 (3.5-11.1)	1.6 ± 0.3 (1.1-2.5) ^a	4.6 ± 0.6 (3.2-5.7) ^b	41.2 ± 5.7 (29.5-49.7)	89.8 ± 3.9 (80.1-99.8)	132 ± 45 (59-204) ^b
>31 ppm (n = 22)	5.6 ± 1.9 (3.8-11.4) ^a	1.3 ± 0.3 (0.9-2.3) ^c	4.2 ± 0.6 (3.5-5.3) ^c	38.8 ± 5.3 (31.6-50.9) ^b	92.9 ± 3.4 (85.7-98.3) ^c	121 ± 43 (65-216) ^a

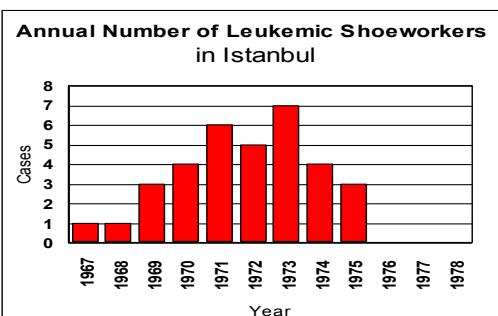
F5.22. Hematotoxicity among Chinese Workers heavily exposed to Benzene.

T5.21. Effects of Exposure to Benzene

Concentration in PPM	Effects
10,000	Death after a few hours
4,000	Narcosis
200-400	Risk of very severe pancytopenias Abnormal blood values in 50-80 % of cases
125-200	Considerable risk of leukemia Severe pancytopenias
65-125	Milder forms of pancytopenias & other cytopenias Acute, headache & fatigue after a few hours
40-65	Hemocytopenia
25-40	Blood cells affected Reduced blood Hemoglobin
10-20	Critical level for leukemia risk
1-10	Chromosome Damage
1	Odor Threshold & OSHA Exposure limit

Before 1950, benzene was responsible for approximately 60 % of secondary (toxic) aplastic anemias, of which 2 to 4 % progressed to acute non-lymphatic leukemia. Benzene is considered to be a human carcinogen, responsible for acute myelogenous leukemia (AML).

In Turkey, where benzene was a common constituent of glues for leather shoes, the incidence of AML climbed to a peak of 7 cases in a study population in 1973. Of some 44 patients with aplastic anemia, 6 died of AML. With introduction of restrictive measures on the use of benzene, the incidence of AML declined within three years to zero (F5.23).



F5.23. Leukemia and benzene exposure.

Occupational levels have been high in the past, but government regulations have forced their reduction to a TWA of 1 ppm.

In a study, Infante and White demonstrated that less than one week of exposure at 10 ppm resulted in chromosomal damage to bone marrow cells, significant depression of bone marrow and disturbances of immune function⁴. It was felt that these observations were highly significant, given the fact that most leukemias and related disorders seem to involve stem cell abnormalities and immuno-deficiencies.

5.3. ImmunoToxicity

5.3.1. Overview of the Immune system

The primary function of the immune system in mammals and lower species is protection from microbial pathogens (bacteria, virus, fungi and yeasts) and tumors.

Lymphocytes play an essential role in immune responses. They are divided into B and T lymphocytes, based on the presence of surface (CD) markers.

T lymphocytes constitute 55-75 % of circulating lymphocytes, and express specific surface markers (CD2 and CD3).

B lymphocytes first develop in the fetal liver, and later in the bone marrow. They constitute 10-20 % of peripheral blood lymphocytes, and express specific surface antigens (CD 21 and CD22).

Antigen-presenting cells (APCs) have the ability to present antigens to lymphocytes in a way that they can be recognized. B lymphocytes recognize self antigens, whereas T lymphocytes recognize antigenic peptides associated with major histocompatibility complex molecules, which includes both self and nonself antigens. The main types of APCs are Langerhans cells, dendritic cells and macrophages.

5.3.2. ImmunoToxicology

Immunotoxicology is concerned with the detrimental effects of foreign substances (xenobiotics) on host immunity, either as a consequence of the activity of a substance or its metabolites on the immune system or as an inappropriate immunological response to that substance. It includes immune responses to xenobiotics that result in allergy and other forms of immune injury.

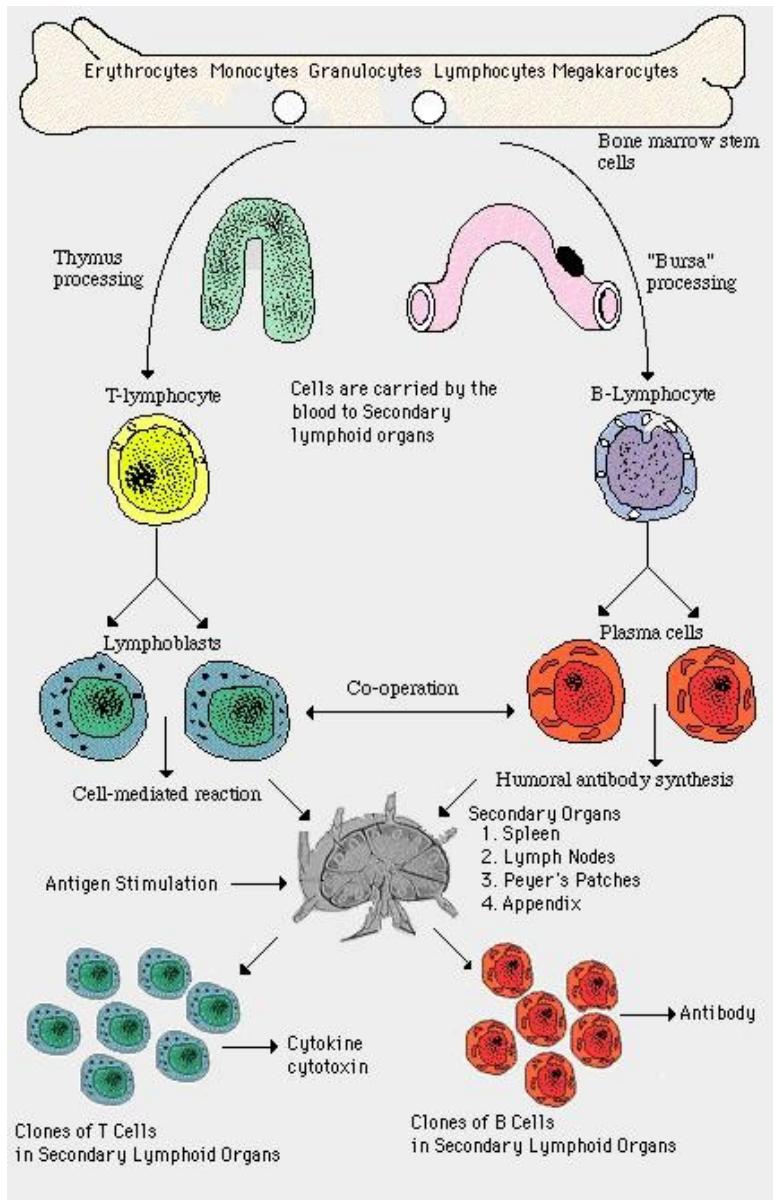
5.3.3. Immune Bioactivation of Xenobiotics

Xenobiotics can modulate the function of the immune system at several levels and can have two opposite consequences: immunosuppression or immune overstimulation. Immunosuppression can result in a diminished resistance against infection, while immunostimulation can result in hypersensitivity reactions, allergies, and autoimmune disease.

5.3.3.1. Immuno-Suppression

A number of xenobiotics can suppress the maturation and development of immune cells and cause immunosuppression. For example, benzene is myelotoxic, because its reactive metabolites can severely damage progenitor cells in the bone marrow, leading to a pancytopenia. This general hematopoietic cell destruction also includes the lymphocyte precursor cells (T and B cells), so most specific immune system cells will be compromised.

Immuno suppression can also be caused by other mechanisms occurring in other parts of the immune system. A widely cited example is the dioxins. The cause of dioxin-induced T cell depletion is found in the thymus, the major site where T cells differentiate. Indeed, after small doses of TCDD, animals exhibit a decrease in thymus mass, leading to thymic atrophy.



F5.24. Immune System.

5.3.3.2. Immuno-Stimulation

In contrast to xenobiotics such as TCDD, which suppress the function of the immune system, other xenobiotics can enhance the function of the immune system. Such an immuno-stimulatory effect can get out of control, and result in harmful reactions. These forms of reactions are rare, but they are toxicologically relevant because the degree of the adverse effects can be severe.

5.3.3.2.1. Hypersensitivity Reactions and Allergies

Two basic forms of such xenobiotic-induced immunostimulation are toxicologically relevant in humans. The first type comprises allergic reactions, which are most commonly known as chemically induced respiratory tract allergies or contact allergies in the skin. In allergic reactions, the immune response is directed against toxicant-modified antigens, involving antibodies and T cells directed against toxicant and antigen.



F5.24a. A worker with protective breathing gear unloads a truckload of barley. The 1000 \$ helmet is battery powered with filters.

5.3.3.2.2. Autoimmune Reactions

Multiple chemical sensitivity (MCS) is an adverse reaction to low levels of many common chemicals. Symptoms may occur after toxicant exposure, by

inhalation or contact. Clinical ecologists believe MCS is a form of immune dysfunction caused by the insidious accumulation of exogenous chemical exposures over a lifetime. Another biologically oriented hypothesis is that MCS represents an atypical biological consequence of chemical injury, or injury to the respiratory tract after an acute inhalational episode. A more recent concept has focused on the relationship between the mucosa of the upper respiratory tract and the limbic (nervous) system.

Autoimmune diseases, such as *lupus*, disproportionately strike women.

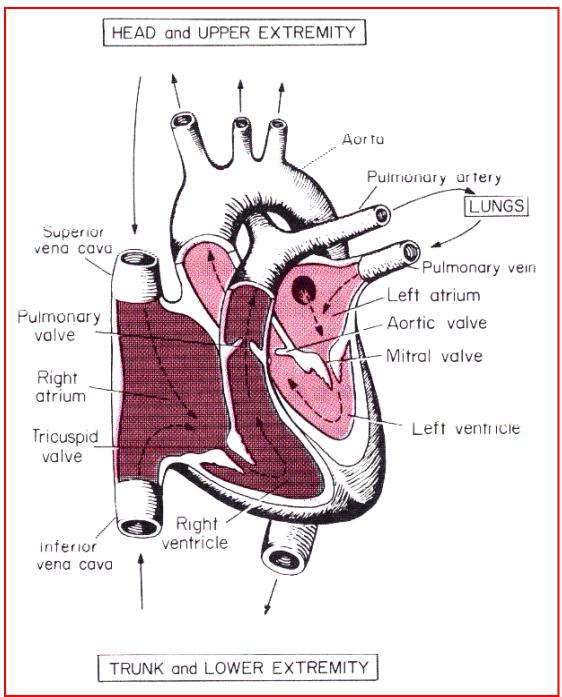
5.4. CardioToxicity

Toxicity of chemicals on the heart must include effects not only on the musculature of the heart (F5.25) but on the nervous tissue that controls the ventricular muscle (Purkinje system), the vasculature of the heart, and the peripheral blood vessels throughout the body.

5.4.1. Cardiac Physiology

The actions of agents on the heart are usually designated as:

- 1) chronotropic effects - relating to "time", a slowing or speeding up of the heart rate,
- 2) inotropic effects - relating to the force of contraction,
- 3) dromotropic effects - relating to nerve fibers and signal conduction.



F5.25. Basic cardiac morphology.
Guyton, 2001.

The major problems encountered in the heart itself usually involve interference either

- (1) with the normal electrical activity of the heart musculature (atria, ventricles), thereby altering regular beating, or
- (2) with the blood supply to the heart, resulting in ischemia (lack of oxygenation), with subsequent damage to the cells, and an interruption of normal electrical flow. Such damage is usually monitored by the electrocardiogram (ECG), specifically the PQRS complex and the T-wave repolarization, with measurements being taken as "point-to-point" and "time-to-points distances" in the ECG to identify the abnormalities (both timing and activity).

Many chemical agents found in industrial settings exert a direct effect on the peripheral blood vessels (constriction or vasodilatation), with subsequent changes in blood pressure causing hypertension or hypotension (severe headaches, dizziness or fainting). Many workers may be on medication

and exposures in the workplace may either antagonize the effects or potentiate them, further confounding the issues.

5.4.2. Chemicals causing Cardiac Toxicity

Many of the *arrythmogens* listed below are also *anesthetics*. Disturbances of the heart rhythm can be life-threatening, but note that occasional heart beat irregularities are also common in health, as shown by Holter monitors: chaotic behavior may be a hallmark of life, with rigidly periodic rhythm being pathognomonic...

Carbon tetrachloride, Chloroform Methyl bromide, Chloropentafluoroethane, Methyl chloride, 1,2-Dibromotetrafluoromethane, Methylene chloride, Dichlorodifluoromethane, Monochlorodifluoroethane, cis-Dichloroethylene, Monochlorodifluoromethane, trans-Dichloroethylene, Octafluorocyclobutane, 1,2-Dichloropropane, Propyl chloride, Dichlorotetrafluroethane, 1,1,1-Trichloroethane, Difluoroethane, Trichloroethane, Ethyl bromide, Trichloroethylene, Ethyl chloride, Trichlorofluoromethane, Fluorocarbon 502, Trichloromonofluoroethylene, 1,2-Hexafluoroethane, Trichlorotrifluoroethane, Isopropyl chloride, Trifluorobromomethane.

T5.26. Arrhythmogenic Halogenated Hydrocarbons.

5.4.2.1. Gases

High concentrations of some gases (Table 5.27) affect hemoglobin in erythrocytes, reducing their oxygen-carrying capacity. Hypoxia ultimately affects the heart. Hypoxia (reduction of oxygen in body tissues below physiological levels) may cause angina (cardiac pain without tissue destruction), arrhythmias, and myocardial infarction (pain with destruction of cardiac tissue). With increasing concentrations

T5.27. Cardiovascular toxicants.

GASES

CO₂, CO, CS₂, CN, H₂S, NO_x, SO₂, etc.

produce anoxia, hypoxia, with effects on hemoglobin and tissues hemoproteins.

ALCOHOLS/ALDEHYDES

ethanol-acetaldehyde, aldehydes in general, dihydroalcohols are cardio-depressants causing negative inotropic effects, cardiomegaly.

METALS

Cd, Pb, Co, Mn, Ba

produce negative inotropic and dromotropic effects as well as structural damage to the heart.

HALOGENATED ALKANES/ALKENES

degreasing solvents, freons, anesthetics (halothane, chloroform), dry cleaning fluids elicit negative chronotropic and inotropic effects, resulting in dysrhythmias, sensitization of cardiac muscle to catecholamines.

ORGANIC NITRITES/NITRATES

cardiac drugs (amyl nitrite, glyceryl trinitrate), room deodorizers (butyl and isobutyl nitrite), explosives (nitroglycerine, trinitrotoluene)

have as a dominant effect vasodilatation of peripheral blood vessels, pooling blood in extremities, production of methemoglobin.

of CO, for example, peripheral vascular effects occur prior to any serious action on the heart².

Industrial scenes are related to incomplete combustion of fuels: furnaces, boilers, automobiles, with hazards increased in cold weather, because of closed doors and windows.

5.4.2.2. Alcohols/Aldehydes

Ethanol (and its metabolite acetaldehyde), dihydroxyalcohols (propylene glycol, polyethylene glycals) and other relatively volatile chemicals cause a *negative inotropic* effect (decrease in force of contraction). Ethanol is effective at 75 mg per 100 ml, just below the "legal limit". Arrhythmias may result from chronic exposure to ethanol, sometimes resulting in ventricular fibrillation and sudden death.

Chronic exposure to industrial alcohols may induce

- (1) cardiomegaly (heart enlargement, dilation of chambers),
- (2) interstitial fibrosis, and
- (3) increased lipids in myocardial cells.

Aldehydes at higher concentrations may stimulate the release of catecholamines, with a sympathomimetic acceleration of the heart rate (positive chronotropic effect).

It is believed that the cardiodi depressant (acute) effects of alcohols and aldehydes are due to an inhibition of intra-cellular calcium transport. Calcium is essential for sodium-potassium transport, inter-cellular communication in the transmission of electrical impulses, and the release of neurotransmitters.

However, aldehydes might stop the formation of *advanced glycation end products*, which would explain the positive effect on cardiovascular health related to wine consumption¹⁶.

Various phenols (phenol and dinitrophenols) are excellent inhibitors of sodium-potassium adenosine triphosphatases

(ATPases). The enzymes pump sodium out of depolarized cells (nerve cells as well as cardiac muscle cells) in order to achieve repolarization. Such agents have a *negative chronotropic* effect and a *negative dromotropic* effect, the heart beating more slowly.

5.4.2.3. Heavy Metals

Lead, cadmium and cobalt have selective cardiotoxic properties, producing *negative inotropic and dromotropic effects*, as well as structural (degenerative) changes in cardiac muscle. The cardiomyopathy seen in drinkers of illicit "moonshine" during prohibition was attributed to lead. The metal originated in the homemade condensing coils soldered with lead, or use of old automobile radiators.

A study in women 65 and older indicates that higher blood lead increases the risk of premature death. Women with less than 8 µg/dl of blood are used as a control, while those with higher lead had a 60 percent increased risk of dying, after accounting for factors such as age, smoking and drinking²⁴.

A bizarre cardiomyopathy affecting habitual beer drinkers in Quebec City was related to the presence of a chemical additive containing cobalt sulphate. The mortality, related to congestive heart failure, was 46.1 %³⁻⁷. A divalent cation, cobalt can substitute for calcium but, perhaps more importantly, it can interfere with hemoprotein (hemoglobin, cytochromes) synthesis, resulting in hypoxic conditions both in the blood and tissues, with impairment of Phase I bio-transforming enzymes (mono-oxygenases).

Other heavy metals (manganese, nickel) can block calcium channels in heart muscle.

Barium is a potent arrhythmogenic agent, as little as 5 mg/kg (intravenous) causing ventricular tachycardia (it is used as a model for screening antiarrhythmic drugs).

5.4.2.4. Halogenated Alkanes/Alkenes

Degreasing solvents, freons, trichloroethylene, perchloroethylene and anesthetics (chloroform, halothane, methoxyflurane) exert similar *negative chronotropic, inotropic, and dromotropic* effects on the heart. Medically, chloroform was the first agent demonstrated to sensitize the cardiac muscle to catecholamines⁸ (epinephrine, norepinephrine) during surgical procedures. Halothane possesses the same property, as do most other halogenated (fluorinated and chlorinated) solvents. The negative inotropism increases with up to 4 chlorines and chlorinated alkenes (trichloroethylene) are more potent than trichloroethanol.

Dysrhythmias result and, if the ECG is done at an appropriate time, one can see skipped beats, irregularly spaced beats, extra beats, all out of phase with the regular electrical activity. The irregularities may be spastic (periodic), necessitating the monitoring of patients for a length of time before the effects are seen. Frequently, the arrhythmias will be related to excessive excitation, which produces an epinephrine "storm", triggering the responses.

Polychlorinated biphenyls (PCBs) are used as electrical insulators, as lubricants, as additives to make plastics more pliable, in adhesives, even as a component of some inks. Being lipophilic, these pollutants settle into fats and oil. David Carpenter of the University of Albany and his colleagues discovered a dose-dependent climb in risk of both systolic and diastolic hypertension with rising blood concentrations of PCBs. Those with less than 1.24 parts per billion were

⁸ Cathecolamines accelerate the heart rate.

designated the reference group. The middle third hosted contaminant levels between 1.25 and 3.64 ppb. Group three: everyone higher than that. The chance of having unhealthy systolic blood pressure — that maximum pressure, which occurs as the heart muscle contracts — was tripled in the middle PCB-contamination group and almost quadrupled in the highest PCB group.²⁶

5.4.2.5. Organic Nitrates

The treatment of anginal pain with amyl nitrite is well known. Crushing a "pearl" of this highly volatile liquid in a cloth and inhaling it produces a rather dramatic relief of pain. Similar effects can be achieved with glyceryl trinitrate tablets placed sublingually and causing a longer action (10 to 30 minutes). By both routes, the heart and coronary vessels receive a relatively high dose of agent which causes localized vasodilatation of the coronary vessels and an improvement of local circulation with a relief of the ischemia. Longer-acting (6-8 hr) organic nitrates (erythrityl tetranitrate, pentaerythrityl tetranitrate) are also used. There are a number of nitrated compounds, particularly in the explosives industry, that cause the same effects.

With restrictions on prescription amyl nitrite, butyl and isobutyl nitrite have been used as room deodorizers in spray applicators, but also as drugs of abuse for enhancing sexual pleasure. The inhalation route produces a "rush". Butyl nitrite has a greater hypotensive effect than lower analogues but not as great as the isobutyl derivative. The toxic effects of nitrite inhalation are shown in Table 5.28, most of them from the peripheral vasodilatation. In addition, nitrites will diminish cardiac contraction without affecting heart rate (decreased efficiency). Other studies noted bradycardia (vagal nerve stimulation) and heart block at higher doses. Exposure has also been associated with tachycardia-hypotension, sometimes

bradycardia-hypertension. Tolerance develops with repeated use, methemoglobinemia becoming a complication.

Fatalities have occurred in workers exposed to organic nitrates after strenuous exercise, 1 to 2 days following cessation of exposure. There is a loss of vascular tone with pooling and trapping of blood in the veins of the lower extremities. Hypertrophy of the left ventricle occurs in workers handling nitro-glycerine (glyceryl trinitrate). Considerable amounts of methemoglobin are formed with chronic exposure.

F5.28. Toxic effects of nitrite inhalation.

Rapid flushing (face)
Pulsation in head, headache
Cyanosis, shallow respiration, respiratory failure
Confusion, weakness, vertigo, fainting, hypotension
Motor unrest
Thready pulse
Clammy skin

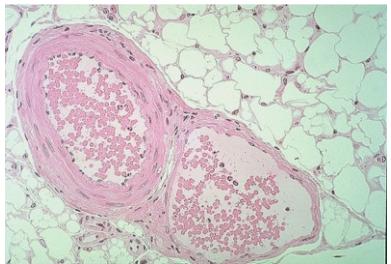
Trinitrotoluene (TNT) is an excellent example of toxicity in the explosives industry. In a Japanese munitions plant, workers complained of dizziness, severe headaches, lethargy, and general malaise. The measurement of blood pressure showed marked decreases in systolic/diastolic ratios before and after work, from lowered systolic pressure. When handling the gel-like mixture, the workers wore surgical rubber gloves, but never changed the gloves, using them day after day, and not discarding them. With a fresh pair of surgical gloves at the beginning of the shift, no change in blood pressure was seen later in the day. Even more impressive was the fact that regularly replaced cotton (washable) gloves protected the workers. The concentration of nitrated compounds were building up over time in the gloves. The compound was being

absorbed by the hot and sweating skin, leading to vasodilatation.

There are a large number of different nitrated compounds used in present-day military and civilian explosives. Miners, re-entering an area in which blasting has taken place, frequently experience severe headaches unless they wait for the ventilation system to clear the area of volatiles. Military personnel found that a "high" could be achieved by putting a small piece of explosive "putty" under the tongue or in the cheek. Nitrate workers can die suddenly after exposure ceases (on weekends and holidays), and may exhibit "Monday morning angina". Re-exposure may trigger rebound vasospasms and fibrillation.

5.5. VASCULAR TOXICITY

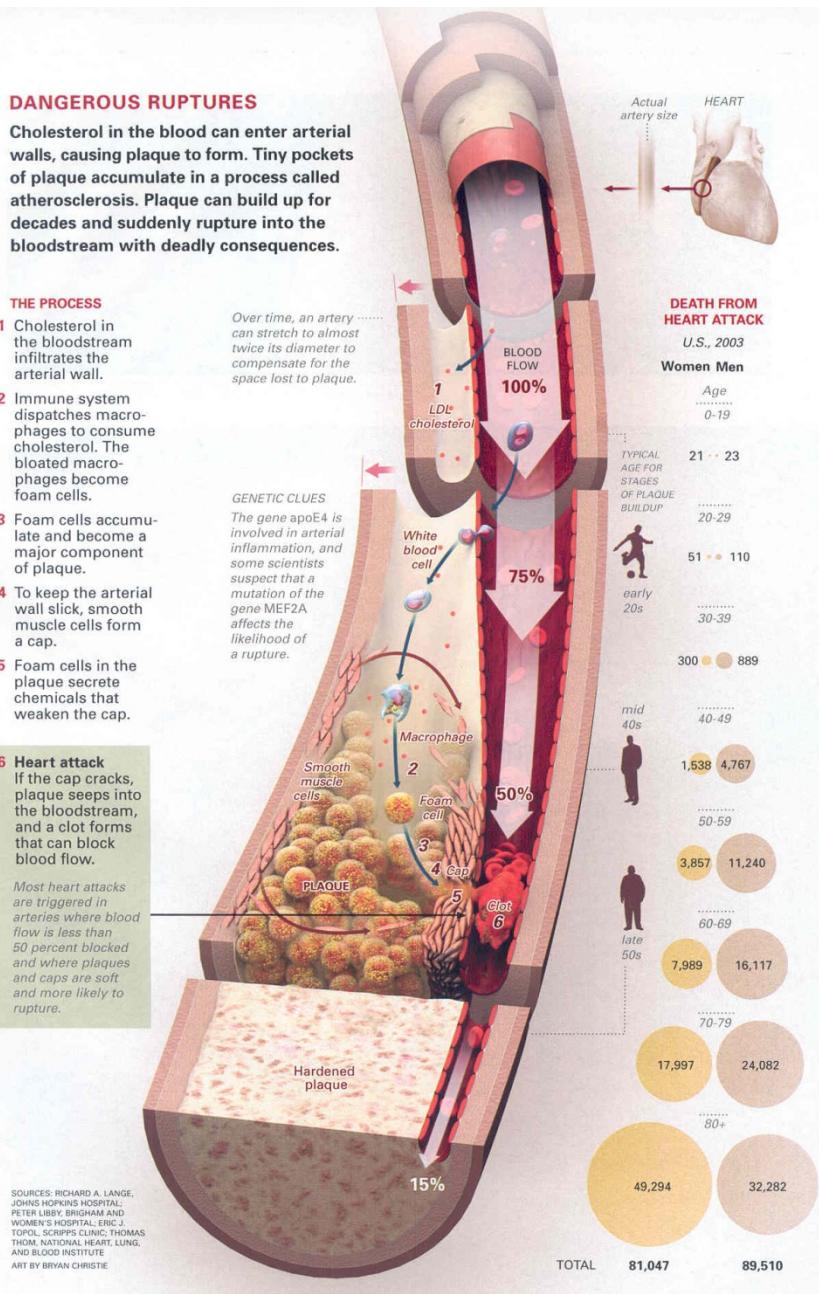
F5.29. Cross section of an artery and a vein. Notice the musculature around the artery.



Since arteries must withhold more blood pressure, and control delivery of blood to tissues, they have a more substantial wall than veins (F5.29). The Smooth Muscle Cells in the arterial wall can be injured after the endothelium has been damaged by a toxicant. In response, they may proliferate, creating a bulge which may be invaded by macrophages. A toxicant may also penetrate through the endothelium to create a mutation in the smooth muscle cells, followed by proliferation. Both mechanisms trigger chronic inflammation and narrowing of the artery. The physiological mechanisms leading to a vascular accident are shown in F5.30.

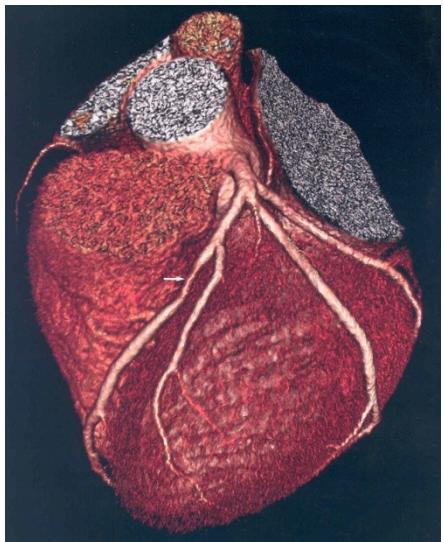
F5.30. Mechanisms of endothelial injury.

National Geographic, Feb. 2007.



Exposure to environmental chemicals can also disable vascular control. Phenanthraquinone, an oily component of diesel soot, inhibits vessel dilatation in older rat muscle, but not in younger ones²².

5.5.1. Atherosclerosis



Some degree of arterial obstructions are found even in young men. Since coronary arteries are not that large (F5.31), it is not surprising that by-pass operations are becoming common.

F5.31. Narrowing of a coronary artery (white arrow).
National Geographic, Feb.2007

It appears that mitochondrial damage foreshadows the onset of atherosclerosis.

Narrowing of arteries due to atherosclerosis can be seen in a beating heart using a 10-second advanced imaging technique (Computed Tomography).

5.5.2. Agents Toxic to Blood Vessels

There are four large classes of agents capable of intoxicating blood vessels: gases, heavy metals, dust particles and others.

5.5.2.1. Gases

Auto exhaust, Carbon monoxide, Nitric oxide, Oxygen, Ozone

5.5.2.2. Heavy Metals

Arsenic, Beryllium, Cadmium, Chromium (deficiency), Copper (Chronic and Acute), Copper (deficiency), Germanium, Indium, Lead, Mercury, Selenium, Thallium

5.5.2.3. Dust Particles

Animal and human studies show that dust particles less than 2.5 µm in air pollution irritate the lungs and provoke inflammation of the blood vessels. Over time, this leads to thickening of the artery walls¹⁷. Long-term exposure to air pollution enhances one's chances of dying from heart attack or stroke. The more pollution there is around your home, the thicker the walls of your carotid artery becomes¹³. Such particles, irrespective of their chemical makeup, are able to suppress secretion of endogenous vasodilators, such as nitric oxide.

Breathing 0.15 µg/m³ of exhaust particles, typical of heavily polluted cities, is capable of immediately rising diastolic blood pressure by 6 mm of Hg. Once the exposure ends, the effect does not last more than a few minutes¹⁸.

5.5.2.4. Others

Allylamine, p-Aminopropionitrile, Boron, Butadiene, Carbamylhydrazine, Carbon disulfide, Chlorophenoxy Herbicides, Dimethylnitrosamine, Dinitrotoluenes, 4-Fluoro-10-methyl-12-benzyanthracene, Glycerol, Hydrogen fluoride, Hydrazinobenzoic acid, Paraquat, Polycyclic aromatic hydrocarbons, Pyrrolizidine alkaloids, Organophosphate Pesticides, T-2 toxin.

Coronary Artery Disease can occur from exposure to volatile carbon disulfide (CS₂) through interaction with a number of enzymes.

A Case of Cardiac Failure

A 51-year old cement finisher spends 2 months spraying and mopping urethane coating in a parking.

He develops rash on his legs and quits because of health concerns. 2 days later, without a known history of heart disease, he is found dead by his wife.

Coroner: cardio-pulmonary arrest, probable acute myocardial infarction.

Two other workers testify of health problems which led them to quit the same job.

MSDS: two part mixture containing polyols, solvents, un-reacted methylene bis-4 cyclohexyl-isocyanate, xylene, 2-ethoxyethyl acetate, un-reacted Toluene Di-Isocyanate.

Measurements show as much as 1 mg/m³ of TDI (7 times the allowed limit). Once sensitized to TDI, individuals can react to concentrations below the odor threshold.

Bronchospasm occurring from exposure to **toluene diisocyanate** can become permanent, resulting in poor lung ventilation. The ensuing hypoxia causes vaso-constriction*, pulmonary artery hypertension and finally right heart failure.

*Vascular reaction in the lung is contrary to reaction in other parts of the body. If vessels are isolated from the lung, they no longer show this reaction (an unknown substance is released by lung tissue).

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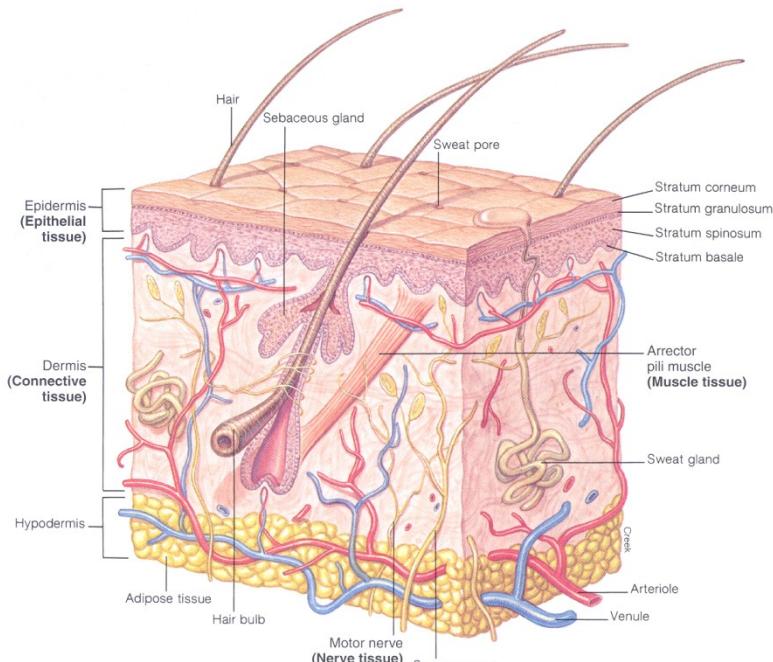
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6. Dermatotoxicity



F6.1. Cross-section of the skin. Fox, 2004.

Skin is the largest "organ", weighing some 3.5 kg (6% of body weight) and covering an area of 2 m² in the average adult. 40% of occupational diseases involve the skin, resulting in prolonged discomfort and expense.

6.1. Skin Anatomy

There are tens of thousands of types of *spiders*, and practically all of them are venomous, because they require venom to kill

and digest their prey. But *only a few* spiders are of lethal consequence to humans, because they possess fangs *large enough to penetrate human skin*.

The skin is composed of three layers (F6.1),

- the *stratum corneum (horny layer)*,
- the underlying *epidermis*, the clear cell layer and
- the subcutaneous complex *dermis* containing nerve endings, muscle fibers, eccrine (sweat) glands, sebaceous glands, hair follicles, connective tissue, lymphatic channels, blood capillaries, lipids, etc.

The *stratum corneum* of dead keratinized cells is the principal barrier layer, composed of high lipid but low water content. The normal role of this layer is to retain water and heat (against dehydration and hypothermia), and to prevent the entrance of microorganisms and most toxic substances.

The *epidermis* is a compacted layer of epithelial cells with tight junctions in the *stratum granulosum*.

The *dermis*, underlying the epidermis, is composed of diverse types of cells including hair follicles, sebaceous glands, eccrine (sweat) glands with a dense capillary bed and good blood flow.

Heat control is managed by perspiration release, cooling of the surface and heat exchange through the capillary bed.

Dermatologists claim that **female** skin is more susceptible to dermatitis, although there is no specific measurement to document this. It seems also that women are more often sensitive to **allergens** as opposed to **irritants**. For example, contact allergy to nickel is found in 15% of women vs 2% of men.

6.2. Skin Penetration

Absorption of chemicals from the skin depends on **surface area** (T6.2), on **skin thickness** (T6.3) and on **exposure** (F6.4). Alcohol in the blood is known to increase toxic compound absorption through the skin. The barrier is imperfect, and substances can penetrate under unfavorable conditions. The efficient absorption in the scrotal area (T6.3, 29%), for example, brings to mind Sir Percival Pott's scrotal cancer cases in young chimneysweeps, the efficient regional absorption of polycyclic aromatic hydrocarbons causing the localized cancers ! The epidermis is replaced about once a month, the stratum corneum about once every two weeks. Therefore, should penetration be exceedingly slow, the toxicant will eventually be shed.

6.2.1. Mechanical

The stratum corneum is breached (by cuts, abrasions or pilar openings). In the occupational setting, workers' hands always have cuts, nicks, and scratches and, in any dermal irritancy test, both intact and abraded skin are tested, since the effects might be quite different. Intact skin is a reasonable barrier whereas "damaged skin" (repeatedly stripped with cellophane tape to remove the stratum corneum) allows 5-fold more benzene to penetrate.

6.2.2. Solvents

Potentially toxic chemical dissolved in an organic solvent (gasoline, oils, trichloro-ethylene, toluene, xylenes, ketones) can penetrate more easily. Rubber solvent, in which traces (0.35%) of benzene occurred, did not enhance the absorption of benzene on single exposure, but did upon repeated exposure or upon application to the palm of the hand⁸.

6.2.3. Aqueous Solutions

Little attention has been paid to the absorption of organic chemicals "dissolved" in aqueous solutions, e.g. wastewater, even potable drinking water used for bathing. The study of a component of gasoline, ethylbenzene, either pure or in aqueous solution, showed that the rate of absorption through human skin was higher for the agent (112 -156 mg/l) in aqueous solution (118-215 µg/cm²/hr) than when applied pure (22-33 µg/cm²/hr)⁴. More recent studies, examining the dermal absorption of toluene, ethylbenzene and styrene in aqueous solutions, representative of drinking water contamination, revealed that the dermal absorption of these contaminants has been consistently underestimated⁵.

Skin penetration of chemicals in aqueous solution tends to decrease exponentially with molecular weight, and to increase with Pow as well as with their water solubility.

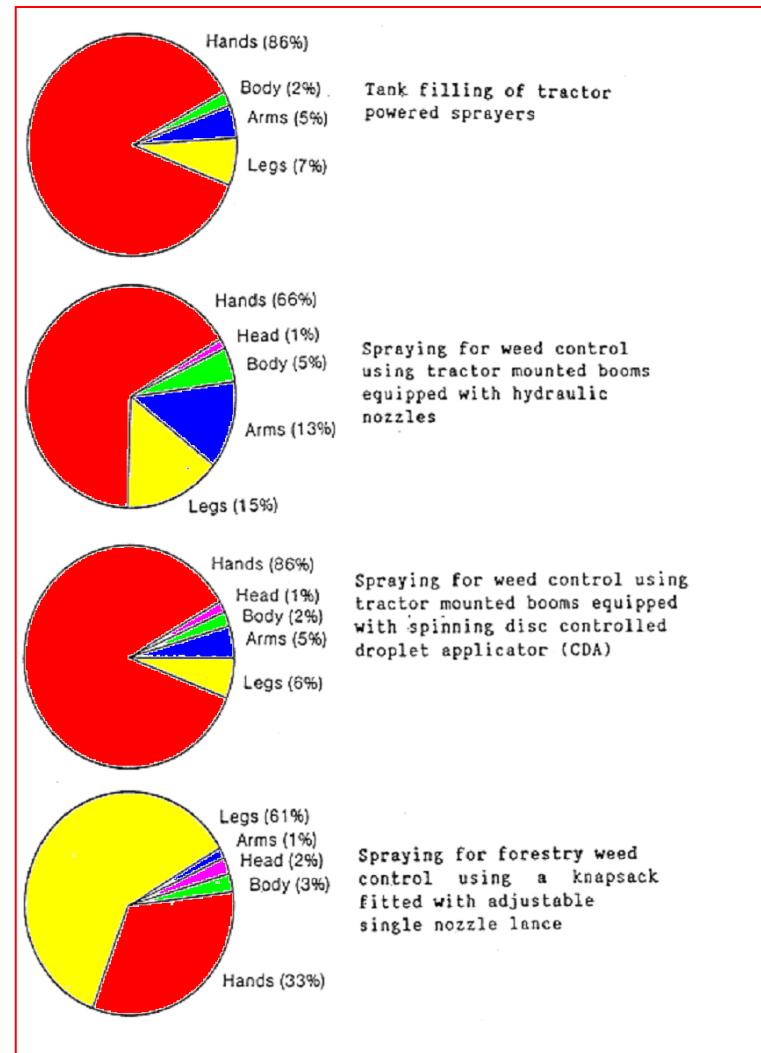
6.2.4. Pesticides

Measurement of parathion absorption in pesticide application (F6.4) reveals that the forearms and hands account for 33 to 86% of the entire dermal dose¹. On the basis of T6.2, T6.3 and F6.4, one could minimize exposure to pesticides by providing solvent-impervious gloves and appropriate headgear. This is what is recommended on many pesticide container labels, in addition to wearing an apron or coveralls while mixing and loading.

These experiments can be extrapolated to industrial chemicals such as benzene, PCBs, and nitroaniline, the results demonstrating a significant acquisition of agent in the epidermis (surface bound to stratum corneum) and dermis, as well as absorbed systemically⁶.

T6.2. Surface of body regions in percent ¹ .	
Body Region	Surface Area (% of total)
Head	5.6
Neck	1.2
Upper arms	9.7
Forearms	6.7
Hands	6.9
Chest, back, shoulders	22.8
Hips	9.1
Thighs	18.0
Calves	13.5
Feet	6.4

T6.3. Urinary excretion of ¹⁴ C-parathion metabolites reflecting absorption rates from different anatomical sites ³ .	
Region of Application	% of dose excreted in urine over 24 hrs
Forearm	1.77
Palm	2.45
Abdomen	3.86
Hand (dorsum)	6.03
Fossa cubitalis (elbow crease)	7.95
Scalp	8.88
Jaw angle	10.92
Post auricular (behind ear)	10.45
Forehead	10.96
Axilla (arm pit)	18.54
Scrotum	28.85



F6.4. Pesticide absorption by region:
% contribution to total dose.

T6.5. Performance of Cutaneous Defense.		
Variable	Black Skin	Caucasian Skin
Abrasion resistance (strippings)	16	9
Stratum corneum layers	21.8	16.7
Density by sucrose gradient centrifugation	1.18	1.11
Thickness (μm)	6.5	7.2
Density (gm/ml)	1.68	1.39
Permeability to water (ml/cm^2 per 24 hrs)	0.4	1.4

6.3. Skin Metabolism

The skin has metabolic functions like any other organ, among which:

1. Synthesis of Porphyrins (cytochromes without the iron) and Heme (respiratory pigments),
2. Production of cytochromes P-450 and P-448,
3. Oxidation of Heme, Alcohols and Aromatic Rings,
4. Alicyclic Hydroxylation,
5. Deamination,
6. Reduction of Carbonyls and C=C,
7. Conjugation: Glucuronidation, Sulfation, Methylation.

Some of the skin enzymes, specifically

- ✚ aryl hydrocarbon hydroxylase (AHH),
- ✚ epoxide hydratase and
- ✚ glutathione-S-transferase,

can be strongly induced by repeated exposure to polycyclic aromatic hydrocarbons and coal tar through the aryl

hydrocarbon receptor. This receptor is known for binding environmental toxicants, but it also regulates the balance of protein and anti-inflammatory responses of the immune system. Exposures to environmental toxicants such as dioxin may encourage the development of autoimmune diseases.

Many of the enzyme activities induced in the skin are directed against invaders such as bacteria and fungi, explaining why some have a toxic component. In the case of many petrochemical exposures, the enzyme AHH transforms chemicals landed on the skin into other compounds of stronger carcinogenic activity.

People who have psoriasis (a hereditary skin disease) cannot be induced for AHH. When exposed to coal tar, these people do not develop higher rates of skin cancer.

Skin tissue is rich in immune cells. Vaccines could be injected into the skin using about 20% of the dose used in intra-muscular injection, and still obtain the same protection. A tight network of cells covering the entire body is formed in the skin by Langerhans cells. These cells ingest antigens present in the skin and transport them to lymph nodes, activating the immune system to protect the body against pathogens.

6.4. Contact Dermatitis

Contact Dermatitis, a most common injury, can be caused by any substance and takes a wide variety of clinical forms:

- ✚ Erythema (reddening of the skin)
- ✚ Purpura (bruise-like)
- ✚ Blistering
- ✚ Eczema, urticaria
- ✚ Rashes (weeping and oozing)

- Erosions of skin
- Hyperkeratosis (thickening, swelling)
- Pustules (small infections)
- Dryness and roughness (scales, itching)

6.4.1. Mechanism of Dermatitis

The effect usually occurs locally on the skin surface, from the degreasing, dehydrating, protein denaturation and osmotic pressure change induced by the toxic agent. The resulting inflammation destroys the stratum corneum, allowing invasion by bacteria and a localized immune response. The condition may be difficult to treat, and persist for some time.

Once through the barrier, the toxicant may penetrate deeper into the subcutaneous layer and exert an effect on the pilosebaceous follicles, resulting in acneiform eruptions, aggravated by hot humid working conditions and exposure to grease and heavy oils.

There is a fair degree of **individual variation** among subjects. If DMSO (a test compound) in a concentration above 80% is put on the skin, stinging is produced in some subjects within minutes, in others within hours, with still others, you give up waiting...

Between 5 and 20% of the population can be classified as "stingers" because of their high susceptibility to many chemicals.

A large variety of compounds (T6.6) produce contact dermatitis. Generally (in increasing order of irritancy),

Alkanes < Alcohols < Aldehydes or ketones < Organic acids < Amines

Some compounds, like anthralin (a psoriasis medicine) induce an irritant response delayed by 8 to 24 hours.

T6.6. Skin Irritants in Industry

Water eliminates natural oils and swells live epidermal cells.

Cleaners solubilize-disorganize barrier lipids and natural moisturizing factors in the stratum corneum, denature protein, intoxicate membranes.

Soaps, Detergents, Bleach.

Oxidants and reducing agents (cytotoxicity, keratolysis).

Plant products, animal protein penetrate the skin through follicular shunts.

Alkalies denature barrier lipids, swell cells.

Inorganic: alkaline sulphides, barium hydrate and carbonate, sodium hydrate, carbonate, silicate and metasilicate, potassium hydrate and carbonate, ammonium hydrate and carbonate, calcium oxide, hydrate carbonate and cyanamide, trisodium phosphate.

Organic: Ethanolamines, Methylamine.

Acids denature protein, induce cytotoxicity.

Inorganic: Arsenious, Hydrofluoric, Chloroplatinic, Hydrofluosilic, Chlorosulphonic, Nitric, Chromic, Perchloric, Hydriotic, Phosphoric, Hydrobromic, Sulphuric, Hydrochloric.

Organic: Acetic, Maleic, Carbolic, Metanilic, Cresylic, Oxalic, Formic, Salicylic, Lactic.

Elements and their salts:

Antimony and salts, Mercuric salts, Arsenic and salts, Nickel salts, Chromium and alkaline chromates, Zinc chloride, Copper sulphate, Silver nitrate, Copper cyanide.

Solvents solubilize barrier lipids, intoxicate membranes.

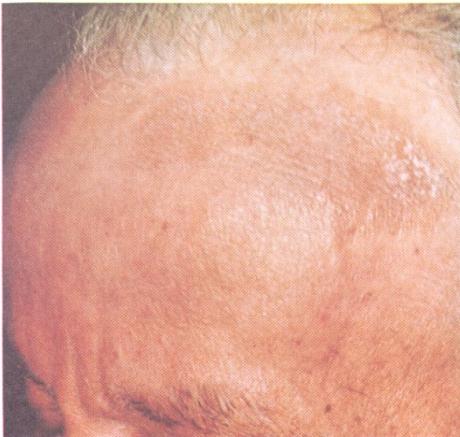
Petroleum solvents, Turpentine, Coal tar solvents, Terpenes, Chlorinated hydrocarbons, Carbon bisulphide, Esters, Alcohols, Ketones.

Acne producers disorganize barrier lipids.

Petroleum oils, Chloronaphthalenes, Cutting oils, Chlorodiphenyls, Pitch, Chlorodiphenyloxides, Tar, Solid chlorobenzols, Paraffin, Solid chlorophenols.

The natural pH of skin being 5.5, substances within the range of 5.5 to 7.4 are usually well tolerated by the skin.

In industry, the activities most likely to be associated with skin diseases are mixing, blending and grinding, painting coating and printing, dyeing, plastic processing, rubber compounding, mining, metal fabrication and woodworking.

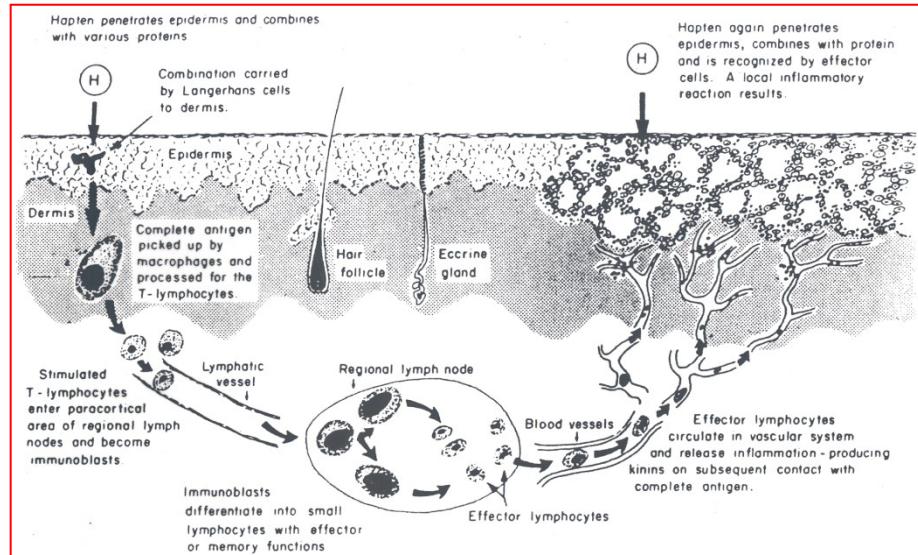


F6.7. Chronic eczematous contact dermatitis caused by leather components in a hatband.

6.5. Allergic Sensitization

Allergic Contact Dermatitis is a cell-mediated reaction (F6.8) that accounts for 30% of all occupational skin diseases. It may, in extreme cases, result in total permanent disability, in part because ordinary protective measures often prove ineffective in view of the small amounts of agent necessary to trigger reactions.

With so-called contact dermatitis "irritants", a previous exposure is *not* necessary, whereas for contact dermatitis "allergy", a previous exposure **IS** necessary. Typical delay in



F6.8. Mechanism of allergic sensitization.

sensitization is 10-21 days, and delay in reaction is 12-48 hours. With successive exposures to an allergen, an individual develops red spots often similar to **eczema** (with or without edema) which appear more rapidly and remain longer in later repeats.

Low molecular weight inorganics can cause allergic sensitization (ex: nickel, chromium in jewelry), but organics such as perfumes can also cause allergy.

95% of substances in perfumes are actually synthetics derived from petroleum. 26 such contact allergens must be identified in perfumes, if marketed in the European Union:

Amyl cinnamal, Benzyl alcohol, Cinnamyl alcohol, Citral, Eugenol, Hydroxy-citronellal, Isoeugenol, Amylcin namyl alcohol, Benzyl salicylate, Cinnamal, Coumarin, Geraniol, Hydroxy-methylpentylcyclohexenecarboxaldehyde, Anisyl alcohol, Benzyl cinnamate, Farnesol, 2-(4-tert-Butylbenzyl)

Propionaldehyde, Linalool, Benzyl benzoate, Citronellol, Hexyl cinnam-aldehyde, d-Limonene, Methyl heptin carbonate, 3-Methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one, Oak moss extract and Tree moss extract.

In digestive allergy, it is known that a larger time to breakdown in the gut for a protein means that it is more likely to be an allergen.

The term “**atopy**” designates an increased genetic susceptibility to the development of occupational dermatitis due to immune cell *hyperreactivity*. Atopic dermatitis is a T-cell mediated, eczematous skin disorder that affects 1% to 3% of adults.

Multiple chemical sensitivity is an adverse reaction to low levels of many common chemicals. Symptoms may occur after toxicant exposure by inhalation or contact. Clinical ecologists believe MCS is a form of immune dysfunction caused by the insidious accumulation of exogenous chemicals over a lifetime. Another biologically oriented hypothesis is that MCS represents an atypical biological consequence of chemical injury, or injury to the respiratory tract after an acute inhalational episode. A more recent concept has focused on the relationship between the mucosa of the upper respiratory tract



F6.9. Appearance of allergic reactions.

European Union Regulations 2010 on Cosmetic Products

Cosmetics include aromatherapy products, but exclude products used solely as medicines. The regulation forbids cosmetic products that may cause damage to human health when applied under normal conditions of use, or reasonably foreseeable conditions of use.

Animal Testing

Forbids testing a finished cosmetic, any of its ingredients or combination of ingredients, on an animal in order that the product may comply with Regulations and the manufacturer must not have tested or commissioned tests on animals of the finished product or its prototype or any of their ingredients. The cosmetic product must also not contain any ingredients that have been tested on animals by others for the purpose of developing new cosmetic products. These restrictions apply where there are authorized alternative methods of test (from 11 March 2013, to tests involving repeated dose toxicity, reproductive toxicity or toxicokinetics).

“Best Before”

If minimum durability is 30 months or less, it must be marked with a 'best-before' date. If minimum durability is more than 30 months, it must be marked with the period after opening for which the product can be used without harming the consumer.

Information available from the 'responsible person'

A responsible person is required to keep certain product information at the registered office address or the address detailed on the product. The product information must include the composition of the product. For perfume or perfume compositions in the product, you are only required to keep the name, code number and supplier identity. Qualitative information for all composites, and the quantitative information in relation to dangerous substances, must also be made easily available to the general public,

- the physico-chemical and microbiological specifications of the raw materials and the finished product, and the purity and microbiological control criteria of the cosmetic product,
- the method of manufacture, which shall be in accordance with good manufacturing practice,
- an assessment of safety for human health of the finished product, including the criteria as stipulated in the Regulations. There are additional criteria where the product is intended for use on children under three years old or exclusively for use in external intimate hygiene,
- the name and address of the person or persons, with the minimum qualifications as detailed in the Regulations, who carried out the assessments,
- existing data on the undesirable effects on human health resulting from use of the product. This information must also be made easily available to the general public,
- evidence to justify any claims made by the product,
- data on any animal testing performed by the manufacturer, his agents or suppliers, relating to the development or safety evaluation of the product or its ingredients,
- there is a procedure detailed in the Regulations that, subject to agreement, allows the confidentiality of some ingredients to be maintained.

and the limbic (nervous) system. Whatever the case, it seems likely that cures for these hypersensitivity reactions will be related to reducing by apoptosis the populations of rogue T-cells.

F6.10. The 1 and 2 Euro coins have been found to release large amounts of nickel in part because they contain two alloys, which create an electrochemical potential. The corrosion is apparent when coins are immersed in artificial sweat (right). Ni triggers allergic reactions¹⁸.



The prevalence of natural rubber latex allergy has been estimated to be 5–18% in health care workers, and latex exposure has been one of the leading causes of occupational asthma in the last several years.

6.6. Chloracne



with comedones (“black heads” from obstruction of sebum evacuation, creating a harbor for bacteria), persistent papules (no pus) or pustules, with a frequent eczema component. Dioxins and Furans (F6.12), among other chemicals, are noted for producing chloracne (F6.12).

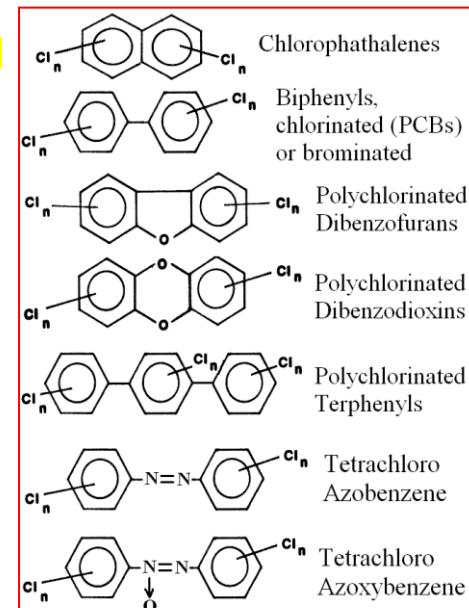
F6.11. Case of Chloracne.

Chloracne is a more severe and specific form of acne which could be labeled *environmental halogen acne*.

Clinically, it is similar to severe *acne vulgaris*,

F6.12. Chloracneogens.

They bind to the AHH receptor, which is also involved, beyond skin metabolism, in a number of developmental pathways. Various vitamin-A derivatives such as tretinoin and retinoic acid have been useful in treating chloracne.



6.7. Skin Carcinogens

The substances currently accepted as Cutaneous Carcinogens are:

- ✚ Ionizing radiation: X-rays, γ -rays, β -rays, energetic particles, protons, neutrons,
- ✚ Ultra-Violet light at 256-320 nm,
- ✚ Physical trauma,
- ✚ Inorganic arsenic,
- ✚ Polycyclic hydrocarbons: Benzo[a]pyrene, 3-methylcholanthrene, dibenzanthracene, 7,12-dimethylbenzanthracene.

Chromium Skin Lesions

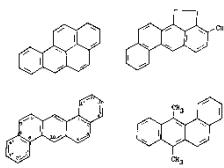
Workers performing chrome plating with exposure to hexavalent chromium from time to time will develop chrome ulcers on their skin (usually hands). Such ulcers have a very distinctive appearance—a “punched out” lesion, frequently with a remaining central core, or ‘umbilicus’, that seems to contain semi-necrotic tissue.

A typical practice would remove the exposure & treat any secondary infections. Healing may take weeks to months.

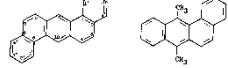
Some experienced platers (several with >30 years in the plating business) self-treat, shelling out the core of necrotic tissue using a crude, non-sterile excision, and they swear that their ulcers heal at least twice as fast.

Another strategy could be to reduce chromium (VI) to the relatively benign chromium(III) within the ulcer using topical ascorbic acid (effective in a guinea pig model).

“Within the chrome plating industry in the West Midlands (Great Britain) some platers treat their chrome ulcers with a substance known locally as 'Black Jack' which is an ichthamol paste applied as an anti-inflammatory agent to the open wound.” Ichthamol is rich in ferrous iron, which would also reduce chromium (VI) to Chromium (III).



Anthracenes are a family of aromatics with 3 benzenes in line (left).



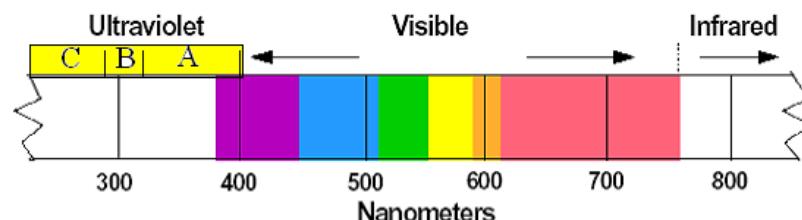
The polycyclic hydrocarbons are found in **soot, pitch, coal tar, creosote; shale, mineral, petroleum and cutting oils**. Petroleum products can be *activated* by skin enzymes or sunlight.

6.8. Barriers

Creams are relatively ineffective against dermatitis, perhaps except for the case of oil-based barrier creams against a wet environment.

6.9. Sunlight

6.9.1. Ultra-Violet Radiation



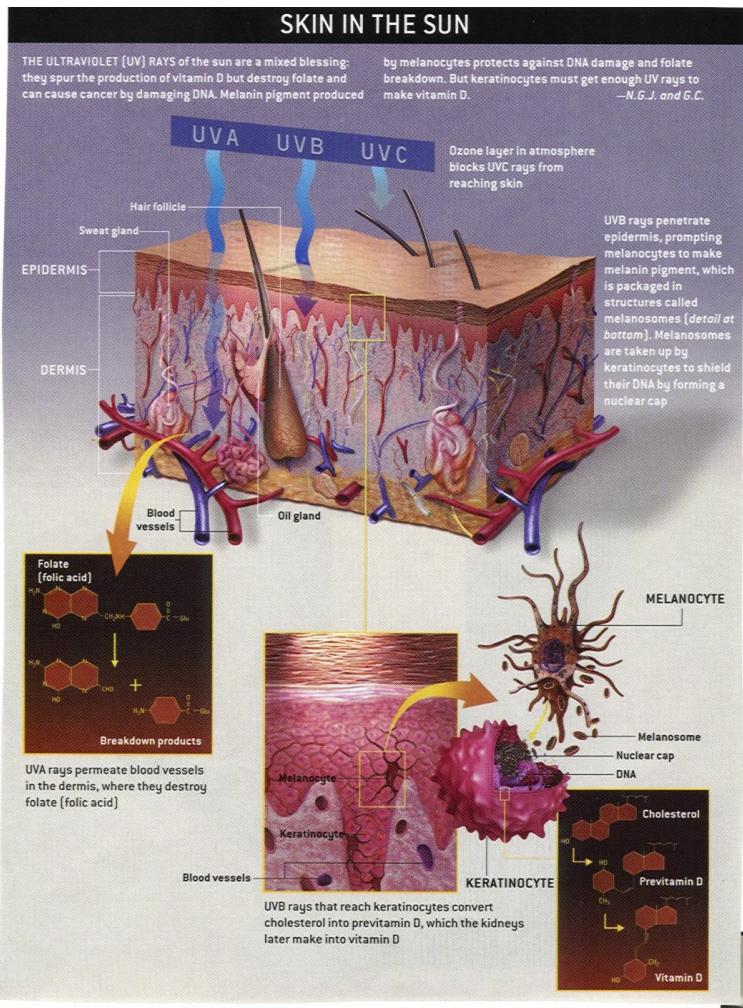
F6.13. “A”, “B” and “C” Ultra-Violet radiation.

Sunlight is distributed according to frequency: infrared (> 700 nm, 49.4%), visible (400-700 nm, 42.3%), UVA (400-320 nm, 6.3%), UVB (320-290 nm, 1.5%), UVC (< 290 nm, 0.5%).

The intact skin is not a barrier to sunlight, particularly to the UVB radiation, the type that causes sunburn.

Sun burning cells using UV radiation triggers Fas-mediated apoptosis of keratinocytes²⁵.

Exposure to UVB when young seems very important in determining the incidence of the various forms of skin cancer later in life. This is why occupational compensation would be limited for such a disease.



F6.14. Penetration of Ultra-Violet Radiation into the Skin.
Scientific American.

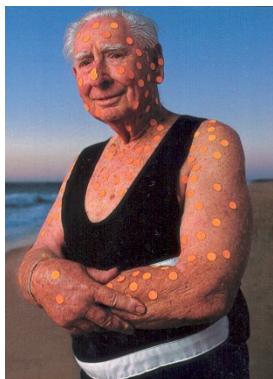
The various forms of skin cancer are:

Melanoma, the deadliest form, involves the pigment-producing cells, melanocytes, from the lower layer of the epidermis. It can develop from a mole or on unblemished skin, grows quickly and can metastasize.

Basal-cell carcinoma: The most common, usually caused by excessive sun exposure. From the lower layer of the epidermis, it develops slowly, rarely metastasizes and is nearly 100% curable if treated.

Squamous-cell carcinoma: from the cells in the upper layer of the epidermis, caused by UV rays, it is usually curable if treated early. Grows faster than basal cell carcinoma and can metastasize.

People with some history of artificial light tanning are 1.5 times more likely to have basal-cell carcinoma and 2.5 times more likely to have squamous-cell carcinoma. If they began before the age of 20, the risks are 1.8 and 3.6²⁵. Young adults are experiencing a sharp increase in non-melanoma skin cancers, probably as a result of full body tanning²⁷.



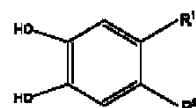
F6.15. A former lifeguard in Australia shows the location of his removed skin cancer lesions (more than 532). Although such cancers are not often lethal, having them correlates with other types of cancers.

National Geographic, November 2002.

Although sunlight is the most frequent cause of skin pigmentation, coal tar and other petrochemical substances may increase pigmentation, while paratertiar

butyl phenols and cathecols (aside) can depigment the skin.

Uneven pigmentation can also occur from heat or microwave exposures.

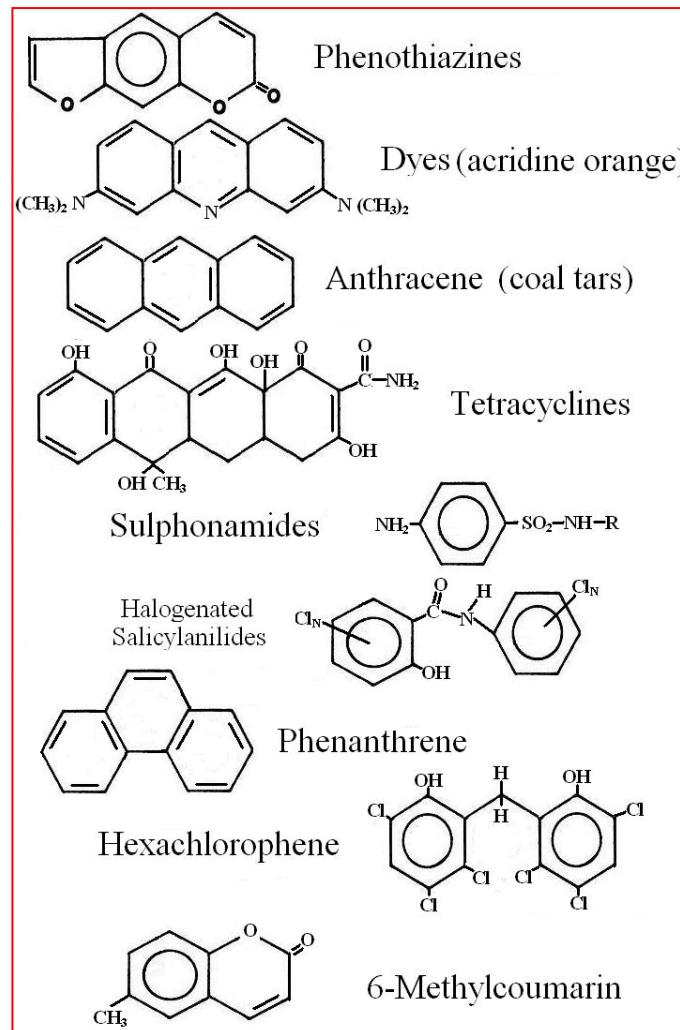


6.9.2. Phototoxicity

In **phototoxicity**, a chemical is converted into dermally toxic compounds by sunlight in the skin.

UVA, 315-400 nm, can activate chemicals into phototoxic or photoallergic agents. The result is similar to contact dermatitis⁷. A phototoxic or photoallergic reaction can be produced with either a dermally acquired chemical or one taken systemically, such as a drug. The first photoreactive chemicals identified were drugs, the sulphonamides, phenothiazines, and tetracyclines. They caused acute dermatitis following ingestion and exposure to sunlight.

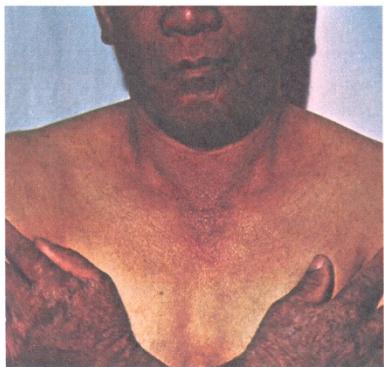
Phototoxicity is usually seen as a delayed erythema followed by hyperpigmentation and desquamation, e.g. a sunburn followed by more severe effects (photo-products with nucleic acids or membrane damage). As can be seen in F6.16, these chemicals usually have large complex ring structures (halogenated benzenes) or interlocking, planar aromatic rings (phenanthrene, anthracene) or multi-rings. Such phototoxicity is independent of immune or allergic mechanisms and can occur in anyone if enough light energy and the concentration of drug in the skin is high enough.



F6.16. Phototoxic chemicals in humans.

6.9.3. Photoallergy

Photoallergy is an acquired, altered reactivity to UV irradiation that is dependent on an antigen-antibody or cell-mediated hypersensitivity response. While relatively uncommon, such reactions are similar to an exaggerated sunburn response and may be immediate (solar urticaria with transient wheal and flare reactions) or delayed (papular and eczematous). Most investigators consider that a cell-mediated (T-cell) immunity is involved, because the characteristic responses observed are similar to that seen in contact allergic reactions, e.g. poison ivy (active ingredient - urushiol). Eventually, as the individual becomes sensitized to the agent, spots will appear at other, often covered, sites on the body where contact with the agent has not occurred. Such conditions have all the appearance of a developing immunity, with time and frequency of exposure being the relevant factors.



F6.17. Photo contact dermatitis often involves areas exposed to the sun.

Since the chemicals that induce such reactions are ubiquitous and may be found in washing powders (brighteners), in perfumes, lotions, creams, etc. (bergamot oil containing bergapten), antibacterials (3,5-dibromo-salicylanilide), emulsifiable cutting oils (petroleum sulphonates, bactericides, rust inhibitors - nitrates and nitrites), it is extremely difficult to identify the toxicant. T6.18 shows a short list of chemicals used in identifying photoallergies.

There are a variety of well known drugs that are photoallergens, and more and more industrial chemicals are showing similar properties.

T6.18. Chemicals used in identifying photoallergy.

Dichlorophene
Bithionol
Hexachlorophene
4,4'-dichloro-3-(trifluoromethyl) carbanilide
3,4,4'-trichlorocarbanilide
3,5-dibromosalicylanilide
3,4',5-tribromosalicylanilide
2,3,4,5-tetrabromosalicylanilide
6-methylcoumarin
Musk ambrette

6.10. Testing

6.10.1. Ocular Irritation: Draize

Accidental eye exposure is a major concern. Irrigation of the eyes is the universal method of treatment for all serious eye exposures. It can be done in a shower, drinking fountain or by blinking in a full sink.

Although the lens includes structural proteins for neutralization of toxins such as glutathione-S-transferase, and heat-shock proteins, it is still very vulnerable.

If an agent is irritative dermally, there is no need to test it in the eye, since eye membranes are more susceptible than the skin's. There is no need to test strong acids and bases, as well as most moderate irritants in the eye. But marginal cases, like a baby

shampoo, where the chance of getting it into the eyes of a child at some time are 100%, should be investigated. If such a product must be safe, how do you test it ?

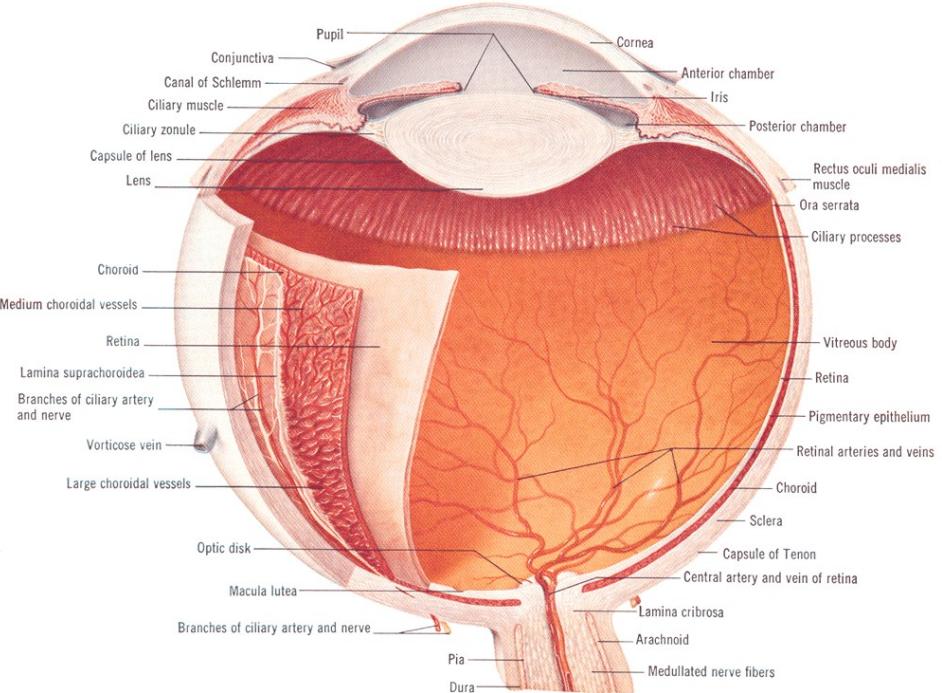
To provide appropriate safety messages on the labels and MSDSs, there is a testing requirement for chemicals. This need was first answered by the Draize ocular test (1944)⁹. It scores the physiological reactions in the cornea, conjunctiva and iris (T6.19). The test is:

- (1) subjectively rated, the simple scoring system varying slightly with methodologies^{9,10},
- (2) vulnerable to interspecies differences.

There appears to be less interspecies variability with strong irritants or with non-irritants; the differences occur mostly with moderate irritants. Draize selected the rabbit as a test animal because it was more sensitive, thereby providing a margin of safety for human exposure², and could display a dose-effect relationship. This test was not satisfactory for all agents; for some, the rabbit eye was not as sensitive as the human eye.

The basic test has changed little in the past 58 years, although smaller volumes ($0.1 \text{ ml} > 0.01 \text{ ml}$) of chemical are now used to reduce adverse effects, while still allowing assessment of potential toxicity.

Various techniques to measure changes in corneal thickness have been tested, specifically a slit lamp perpendicular to the corneal apex. The measurements are accurate to 0.01 mm⁴. Five measurements are made at each time point (0, 24, 48, and 72 hr post-treatment).



F6.19. Anatomy of the lower half of the eyeball.

6.10.2. Dermal Irritancy

In view of the frequency of accidental splashes and spills, it is important to have MSDS data on the irritating or corrosive effects of a chemical on the skin. Again, the most successful test (T6.20) was proposed by Draize⁹.

Most suitable animals possess a good fur coat which can be shaved, leaving only a fine stubble. There are strains of mice and guinea pigs that are hairless or "nude": sad looking, wrinkled things that generate sympathy. There is no agreement on which skin is morphologically closest to the human skin.

T6.20. Eye irritation test: grading ocular lesions.

From Draize, J. H., Woodard, G., and Calvery, H. O., *J. Pharmacol. Exp. Ther.*, 82:377-390 (1944).

DESCRIPTION OF LESION		#
I. CORNEA: A. Opacity, degree of density		
Scattered or diffuse areas of opacity, but details of iris clearly visible	1	
Easily discernible translucent areas, details of the iris slightly obscured	2	
Opalescent areas, no details of iris visible, size of pupil barely discernible	3	
Opaque cornea, iris invisible through opacity	4	
I. CORNEA: B. Area of cornea involved		
One quarter (or less), but not zero	1	
Greater than one quarter	2	
Greater than one half	3	
Greater than three quarters, up to complete area	4	
Score = Part A x Part B x 5 --- maximum score = 80		
II. IRIS: A. Normal		
Folds above normal, congestion, swelling circumcorneal injection, iris light-reacting	1	
No reaction to light, hemorrhage, gross destruction	2	
Score = Part A x 5 ---- maximum score = 10		
III. CONJUNCTIVA: A. Redness (refers to palpebral conjunctiva)		
Blood vessels hyperemic (injected)	1	
Diffuse, crimson color, individual vessels not easily discernible	2	
Diffuse vessels, beefy red	3	
III. CONJUNCTIVA: B. Chemosis (lids and nictitating membranes)		
Any swelling above normal (includes the nictitating membranes)	1	
Obvious swelling with partial eversion of lids	2	
Swelling with lids half closed	3	
Swelling with lids more than half closed	4	
III. CONJUNCTIVA: C. Discharge		
Not normal (ignore small amounts observed in inner canthus of normal animals)	1	
Discharge with moistening of lids and hairs adjacent to lids	2	
Discharge with moistening of the lids and hairs of considerable area around eye	3	
Score = (Parts A + B + C) x 2 --- maximum score = 20		

Investigators use rodents, guinea pigs and rabbits, all of which have sensitive skin. Larger animals such as swine, minipigs, and dogs may also be used. For a wide range of chemicals, the skin of the rhesus monkey is considered to best mimic the

human skin. However, dermal irritation studies are usually conducted in a specific animal species for simple practical reasons, selection usually having little to do with any similarity to the human skin.

T6.21. Skin irritation test: grading skin lesions.

Skin responses	Value
Erythema and Eschar Formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate-to-severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injury in depth)	4
Edema Formation	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well-defined by definite raising)	2
Moderate edema (raised approximately 1.0 mm)	3
Severe edema (raised more than 1.0 mm and extending beyond the area of exposure)	4

Two effects are observed on skin during the 24, 48 and 72 hours post-exposure: *erythema* (redness) and *edema* (puffiness or swelling).

It comes down to how red is red and to what degree is swelling seen. A numbering system or grading score has been developed for the two parameters which is dependent upon the skill of the investigator or technician to "grade" the severity of the biological response (See T6.21).

Having scored each animal for erythema and edema, obtained average scores for each parameter and incorporated them together, one is left with interpreting them for the products.

A scheme used by NIOSH is shown in T6.21. Based on these ratings, numbers generated in the test can be converted into label warnings and safety information in the MSDS (T6.22). In the case of *dermal sensitization* studies, the animal of choice is usually the guinea pig, although rabbits can be used,

both being reactive and sensitive. Essentially, the chemical of interest is applied to the skin daily over a 14-day period and, following a resting period of 10-to-14 days, the animals receive a challenge dose of the agent, usually a lower concentration than was applied earlier. A positive flare-and-wheal reaction, usually dose-dependent, signifies that *sensitization* has occurred.

T6.22. NIOSH interpretation of skin testing ratings.

McCreesh and Steinberg, 1983

Rating		Interpretation
Intact Skin	Abraded Skin	
0-0.9		Nonirritant: probably safe for intact human skin contact
1-1.9		Mild irritant: may be safe for use but appropriate protective measures are recommended
2-4.0		Too irritant for human skin contact: avoid contact
	0-0.9	Nontoxic to cellular components of abraded skin: probably safe for human skin contact
	1-1.9	Mild cellular toxins: may be safe for abraded skin contact
	2-4.0	Cellular toxicant too irritant for abraded skin contact: avoidance of contact is advised

6.10.3. Irritation-Corrositex vs Draize

The descriptions of T6.20 and T6.21 have attracted the attention of animal rights activists, who have campaigned to eliminate such studies on the basis that they inflict needless

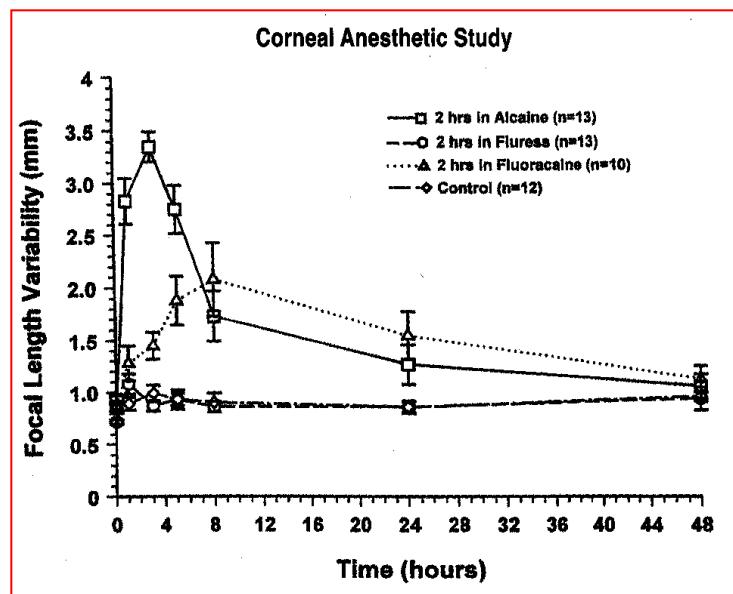
pain, and use too many animals because of the variability in response.

6.10.3.1. Substitute Ocular Tests

Many promising alternative **ocular tests**, some of which are being accepted by regulatory agencies, have been developed, including isolated cornea, enucleated eyes, cultures of corneal and lens cells as well as non-animal preparations¹¹. This still leaves the problem of a suitable species from which to obtain the eyes for the *ex vivo* tests. The results of a comparative study sponsored by the Commission of European Communities revealed that the enucleated chicken eye appeared to be the most practicable when compared with the eyes of swine and cattle and tested with a number of well known chemicals¹⁵. The Chicken Enucleated Eye Test (CEET) recognizes three levels of irritancy. Since large numbers of chicken eyes can be obtained at little cost from slaughterhouses, this test would appear to satisfy animal rights groups and still give a valid assessment of ocular toxicity. A competing technique uses isolated (enucleated) eyes following euthanasia of the rabbit, incubating *in vitro* (or *ex vivo*) with the test toxicant, with corneal thickness measurement with the slit lamp¹². Comparing the *ex vivo* and *in vivo* tests has demonstrated that materials capable of causing ocular injury caused an intensification of the stromal image and swelling of the cornea with a good correlation between *in vivo* ocular damage and *ex vivo* results¹⁴. Since animal lenses can be cultured over days or weeks with their optics and repair mechanisms intact, even chronic changes as well as recovery can be documented. Variables used to gauge toxicity can be as simple as changes in the focal length of the lens, as shown below for three common corneal anesthetics.

6.10.3.2. Substitute Skin Tests

The development of synthetic **skin tests** has also been intensive. “Corrositex” is an *in vitro* test that determines chemical corrosivity and allows assignment to packing group specifications. It replaces the rabbit skin test. It is based on a glass vial filled with a chemical detection system, and capped



F6.23. Effect of focal length of bovine lens from three eye anesthetics.

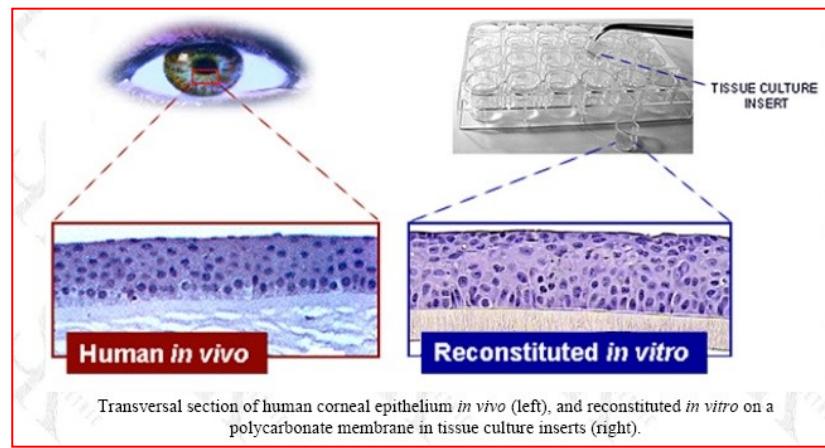
by a bio-membrane. After a potentially corrosive chemical is placed on the membrane, the chemical detection system may become colored.

“Irritation” is a quantitative *in vitro* test that ranks ocular and dermal irritation potential of cosmetics, consumer products, pharmaceuticals and raw industrial chemicals.

The chemical under investigation is placed on a membrane that permits controlled delivery of the material to a reagent

solution. The reagent solution contains proteins, glycoproteins, lipids and low molecular weight components that self-associate to form insoluble matrices. The toxic changes are measured over 5 hours using turbidity readings.

Most of the new *in vitro* tests offer considerable simplicity, economy and speed. Some things are lost from the animal models. The opportunity to observe many variables (as opposed to only a change in color or turbidity), to make chronic observations over time (as opposed to a few hours). Certainly the new tests are simpler to interpret. But simplicity of interpretation goes in hand with paucity of output information. The usefulness of the tests depends on how carefully they are designed and researched.



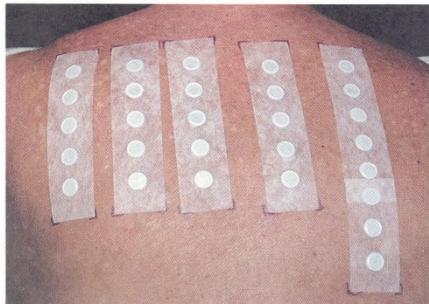
Transversal section of human corneal epithelium *in vivo* (left), and reconstituted *in vitro* on a polycarbonate membrane in tissue culture inserts (right).

F6.24. When cultivated at the air-liquid interface in chemically defined medium, the transformed human corneal epithelial cells of the cell line HCE form a corneal epithelial tissue (mucosa), devoid of stratum corneum, resembling ultrastructurally (tissue morphology and thickness) the corneal mucosa of the human eye.

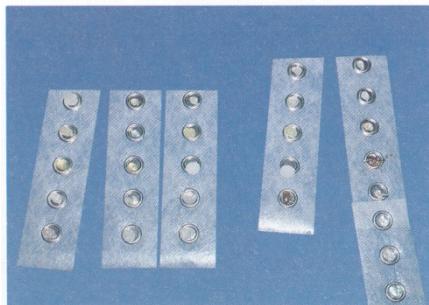
6.10.4. Clinical Patch Tests

People may need to be tested for their reaction to chemicals. Allergens are tested either in a petrolatum or aqueous vehicle in Finn chambers (F6.25), essentially a filter paper used to contain water preparations, The patches are maintained on the skin for 2 days. Reactions are graded one hour after the tape is removed. They are normally conducted with non-irritant concentration, to determine an hyper-sensitivity specific to a patient.

When there is greater reaction at the edge of the patch, this usually denotes irritation rather than allergy. Depigmentation can sometimes result from exposure to test chemicals. Sub-cutaneous injections (F6.26) can also be used.



F6.25. Finn chambers for allergic sensitivity tests in patients.



F6.26. Allergic sensitivity tests using sub-cutaneous injections.

The large number of chemicals that can be used in various versions of the clinical patch tests speaks to the variety of possible cutaneous reactions.

F6.27. Chemicals used in diagnostic patch testing. Unless noted, chemicals are in petrolatum in the first two columns. Chemicals present in more than one column are in bold.

	North American Contact Dermatitis Group	European Contact Dermatitis Group	Thin Layer Rapid-use Epicutaneous Test (allergens)
1	Benzocaine, 5%	Potassium dichromate 0.5%	Nickel sulfate 0.2 mg/cm ²
2	Mercaptobenzothiazole, 1%	Paraphenylenediamine base, 1%	Wool alcohols 1.00 g/cm ²
3	Colophony, 20%	Thiuram mix, 1%	Neomycin sulfate 0.23 mg/cm ²
4	Paraphenylenediamine base, 1%	Neomycin sulfate, 20%	Potassium dichromate 0.023 mg/cm ²
5	Imidazolidinyl urea, 2% aqueous	Cobalt chloride, 1%	Caine mix 0.63 mg/cm ²
6	Cinnamic aldehyde, 1%	Benzocaine , 5%	Fragrance mix 0.43 mg/cm ²
7	Lanolin alcohol, 30%	Nickel sulfate, 5%	Colophony 0.85 mg/cm ²
8	Carbamix mix, 3%	Clioquinol, 5%	Epoxy resin 0.05 mg/cm ²
9	9. Neomycin sulfate, 20%	9. Colophony, 20%	Quinoline mix 0.19 mg/cm ²
10	Thiuram mix, 1%	Paraben mix, 8%	Balsam of Peru 0.80 mg/cm ²
11	Formaldehyde, 1% aqueous	N-isopropyl-N-phenylparaphenylenediamine, 0.1%	Ethylenediamine dihydrochloride 0.05 mg/cm ²
12	Ethylenediamine dihydrochloride, 1%	Wool alcohols, 30%	Cobalt dichloride 0.02 mg/cm ²
13	Epoxy resin, 1%	Mercaptomix , 2%	p-tert-Butylphenol formaldehyde resin 0.04 mg/cm ²
14	Quaternium 15, 2%	Epoxy resin, 1%	Paraben mix 1 mg/cm ²
15	p-tert-Butylphenol formaldehyde resin, 1%	Balsam of Peru, 25%	Carbamix 0.25 mg/cm ²

16	Mercaptomix, 1%	p-tert- Butylphenol formaldehyde resin, 1%	Black rubber mix 0.075 mg/cm ²
17	Black rubber mix, 0.6%	Mercaptobenzothiazole, 2%	Cl+Me-isothiazolinone 0.0040 mg/cm ² (Kathon CG)
18	Potassium dichromate , 0.25%	Formaldehyde, 1%	Quaternium 15 0.1 mg/cm ²
19	Balsam of Peru, 25%	Fragrance mix, 8%	Mercaptobenzothiazole 0.075 mg/cm ²
20	Nickel sulfate, 2.5%	Sesquiterpene lactone mix	Paraphenylenediamine (PPD), 0.090 mg/cm ²
21		Quaternium 15, 1%	Formaldehyde 0.18 mg/cm ²
22		Primin, 0.01%	Mercaptomix 0.075 mg/cm ²
23		Cl + Me-isothiazolinone, 0.01% aqueous	Thimerosal 0.0080 mg/cm ²
24			Thiuram mix 0.025 mg/cm ²

6.11. Case Study: Carbamate and Propanil Pesticide case and Chloracne

(modified from Williams & Burson)



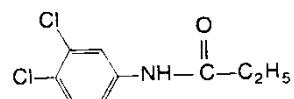
A plant in Arkansas manufactured several types of pesticides in rotation, manufacturing and packaging one pesticide for several weeks or months, and then doing the same with another pesticide, and then perhaps a third. The CDC became involved at this plant because there had been several hospitalizations of workers and also a number of complaints. OSHA inspected the plant and requested that a health hazard evaluation be done. Two physicians from the CDC then surveyed the plant. On investigation, they found that two types of compounds were produced.

One compound was a carbamate, methomyl (N-[(methylcarbamoyl)oxy] thioacetimidic acid methyl ester) which causes symptoms of toxicity similar to those caused by organophosphorous compounds—namely, nausea and vomiting, small contracted pupils, increased salivations, and muscle fasciculation. Methomyl is a highly toxic carbamate.

In addition to the methomyl, the plant was making and packaging an herbicide called propanil (3',4'-dichloropropionanilide), made from 3,4-dichloro-aniline. A number of employees had signs traceable to propanil: "acne," which was really chloracne (see figure), and also a nonspecific rash and skin irritation. These skin problems were more of a problem than the toxic effects of the methomyl. In trying to evaluate the problems, we divided the workers into different groups. There were some differences in symptoms, but because the chemicals were so pervasive, it was

not possible to demonstrate that very well. Workers also moved freely from one area into another, and many had been exposed to both compounds.

The herbicide propanil, which is an aniline-type chlorinated compound, has the following structure:



Propanil has a number of different commercial names. It is not very toxic, but it does cause the skin disease chloracne, which can be quite persistent and disfiguring. Propanil has this effect because it is contaminated with 3,4,3',4'-tetrachloroazoxybenzene. This compound is chloracnegenic because of a peculiar chemical configuration, in which two chlorine atoms on one aromatic ring are connected either by another ring or by a double bond to two chlorines on another ring, reminiscent of TCDD (dioxin), 2,3,7,8-tetrachlorodibenzo-p-dioxin, which is extremely toxic. It has only recently been established that the chloroazobenzenes also cause chloracne. Other herbicides—diuron, linuron, neburon—may also be contaminated with tetrachloroazoxybenzene. Other chemicals that cause chloracne are the chlorinated naphthalenes, biphenyls, dibenzodioxins, and dibenzofurans. In a few documented cases patients have had active chloracne for up to 20 years after they were exposed to one of these chemicals. In many instances chloracne gradually improves over a number of years following cessation of exposure. Until we made our investigation it had not been known that propanil caused chloracne. Our findings were substantiated by measuring chlorinated azobenzene in the propanil and by testing the chloracnegenic effect of this material on the rabbit ear.

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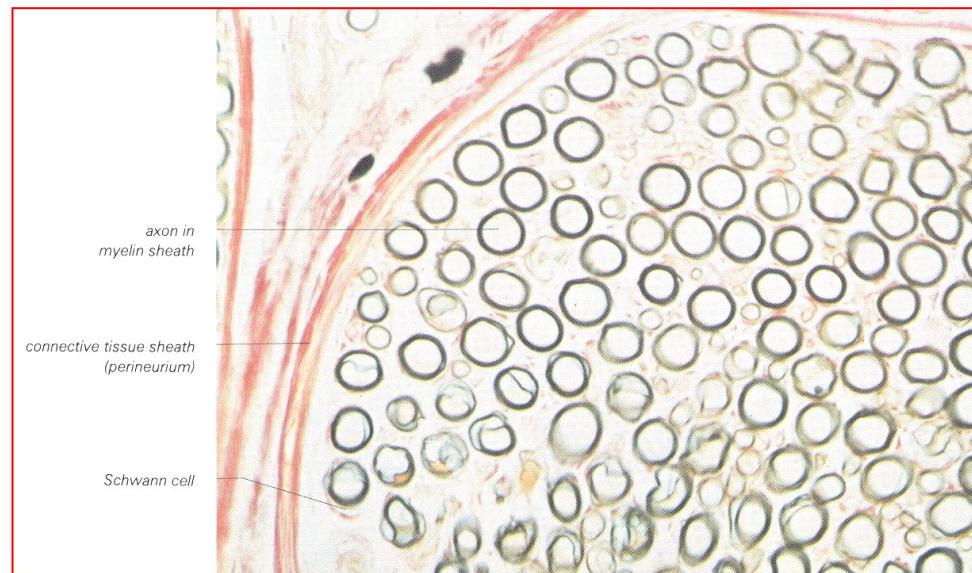
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Neurotoxicity

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7. Neurotoxicity



F7.1. Transverse section of a nerve. X700. The red tissue is a nerve sheath. The dark circles are nerves wrapped with myelin, the smaller light ones are unmyelinated nerves. *Biological Structures.*

7.1. Anatomy

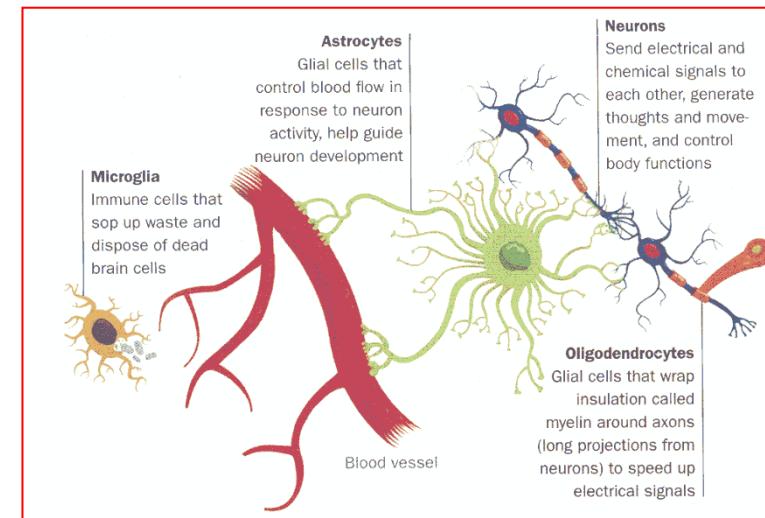
The nervous system includes the brain, spinal cord, and the peripheral sensory and motor nerves. The *central nervous system* is the brain and spinal cord only.

Nerve cells normally grow when they are young, and stop when they are mature. Injured central nervous system cells are mostly unable to regenerate on their own (they do so to a very limited degree; there are very few absolutes in biology). In the peripheral nervous system cells, however, an injury can

stimulate the cells to regrow, making it possible for severed limbs to be surgically reattached to the body, and regain function.

In the brain and spinal cord, *white matter* corresponds to the thick protective *myelinating sheaths* around nerves and nerve bundles. The *gray matter* is made of cell bodies and nonmyelinated or lightly myelinated fibers.

In higher vertebrates, the proportion of white to gray matter increases as one ascends the phylogenetic tree until, in man, more than 40 % of the cross-sectional area through the brain consists of glistening myelin.



F7.1a. Types of cells in the central nervous system.

There are various cell types in the brain, and the neurons have been the star, primarily because of their spectacular action potentials (F7.3). Neurons cohabit the brain with glia, which outnumber neurons by a factor of 10. This has led to the common misconception that humans “use 10 % of their brain”,

underlining the opinion that glial cells contribute nothing to thinking. Specialized glial cells called *astrocytes* influence connections between neurons, the level of chemical messengers in the synapse, as well as the blood flow within the brain. They collect nutrients from the bloodstream, and purify them for neurons, protecting the brain from some toxic agents. When this protection fails, for example for solvents which can easily gain access to brain tissues, a variety of effects occur, some of which are reversible (for example, alcohol intoxication).

In humans and in many animals, a long childhood implies an adaptable neural structure that must be programmed through experience primarily in childhood, but even later in life. The processes needed for proper programming of such a complex structure probably increase the vulnerability of the brain to toxicants (lead is a good example).

T7.2. Physio-pathology of neuronal damage.

Vulnerabilities

- Post-mitotic cells (little cell division)
- Poor regeneration
- Very vulnerable to oxygen depletion
- Reasonable blood flow (toxicant access)
- High lipid content (hydrophobic substances)
- Unique shape and structure (long neurons)
- Excitable membranes (receptors)
- Low energy stores (glucose needed)
- Axonal transport of nutrients (long distance)
- Specific neurotransmitters (vulnerable)
- Myelination

Protective mechanisms

- Extra neurons (safety factor)
- Local reorganizations (brain and peripheral plasticity)
- Adaptive responses (re-programming)
- Blood-brain barrier (denying access to toxicants)

The nervous system is a frequent target for a variety of chemicals: metals, solvents, plastics, monomers, agrochemicals... The protective mechanisms are limited, and damage often poorly reversible. The reasons for the nervous system's vulnerability are itemized in T7.2.

7.1.1. Blood-Brain Barrier

The endothelial cells of the vessels in the central nervous system are held together by *tight junctions* between cells, preventing access of unwanted substances. The cells of the barrier also have larger numbers of mitochondria and low pinocytotic activity.

The barrier is successful in preventing the following toxicants from entering: lead compounds, penicillin and staphylococcus toxin. But lipid-soluble compounds such as methyl mercury, pentachlorophenol, hexachlorophene, trimethyl tin and triethyl tin can penetrate cell membranes, and make it through the blood-brain barrier. The blood-brain barrier, like the Great Wall in China, is not continuous, but has gaps in the hypothalamus and the choroid plexa. In fetuses and very young animals, the barrier is under construction.

There is a vulnerable portal through the nose and the olfactory bulb that even allows small ($0.1 \mu\text{m}$) airborne particles into the central nervous system. Contrary to the lungs, the particles are not easily cleared from the CNS¹³.

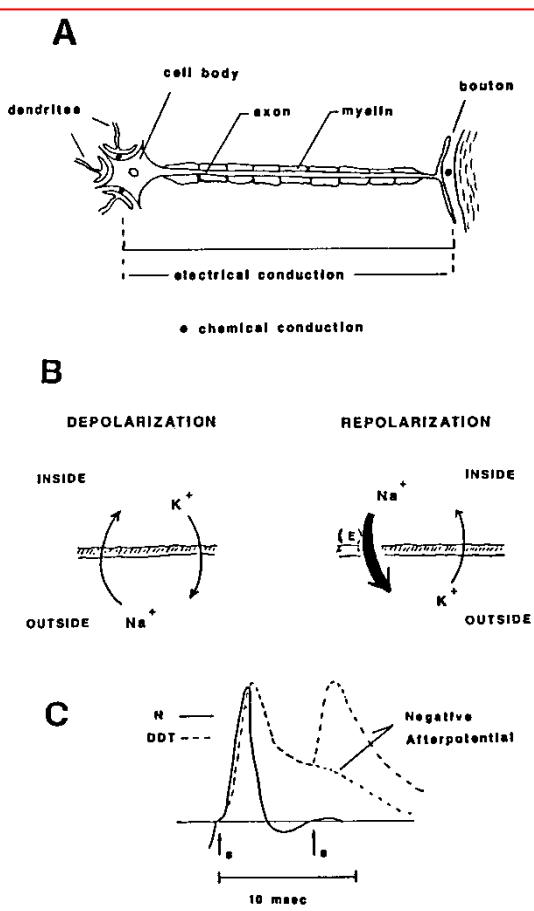
Mannitol opens the blood-brain barrier.

7.1.2. Electrophysiology

The basic structure and polarization of a neuron are depicted in F7.3. Anatomically, there is a cell body with dendrites, making close contact with the terminal portions of adjacent neurons. Communication between one neuron and another (across a synaptic cleft or gap) is chemical in nature, requiring specific *neurotransmitters* released from the terminal to a receptor.

Agonists substitute for naturally occurring *neurotransmitter* substances and produce a response.

Antagonist substances bind to receptors *without* producing a response.



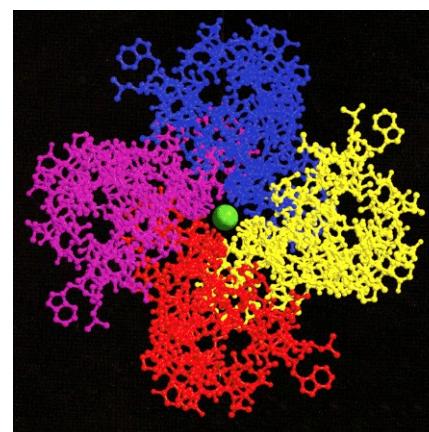
F7.3. Polarization of neurons.

Attachment of the neurotransmitter to a sensitive "patch" (receptor) on the cell body of the next neuron results in depolarization of the cell membrane and the propagation of an electrical impulse down the axon of the neuron.

With depolarization, there is an opening of pores in the cell membrane, permitting the influx of large quantities of sodium ions, with a corresponding loss of potassium ions to the exterior of the neuron (F7.3B) and a loss of electrical potential from a standing -80 mV to 0 mV (F7.3C).

With repolarization of the neuron in preparation for another stimulus, the open pores (gates) close, the internalized sodium is pumped out by specific enzymes ($\text{Na}^+ - \text{K}^+$ ATPase), allowing an influx of potassium and accompanying chloride ions.

All of this, under normal conditions takes no more than 10 milliseconds, but if the ability to repolarize is impaired (as with the insecticides DDT and pyrethrins), one sees a slow repolarization with a prolonged negative after potential (F7.3C), a state in which the nerve is only partially repolarized and is highly susceptible to rapid depolarization again.



F7.4. The specificity of ion channels is a size-matching and electrostatic effect. Here, a potassium ion (green) sits at the center of a bacterial ion channel (KcxA). The channel lets in one sodium for each 1000 potassium ions, stripped of its hydrating molecules.

7.1.3. Myelin

If you hit your finger with a hammer (you may try this at home), you may notice that the feeling of pain will "reach you" later than the hit detection. This is because these two signals are not propagated by the same neuron size.

The depolarization-repolarization is relatively slow in non-myelinated nerves, each small segment of neuronal membrane having to undergo the event as the pulse propagates down the

axon. To increase this velocity, a size increase proportional to the square root of the diameter of the neuron is required. So, if the rate of conduction is to be doubled, the nerve must be four times larger in diameter. This would lead to very large nerves when high speeds are needed.

A coating of *myelin* over axons reduces linear capacitance of nerves by 1000 and increases resistance by 5000, substantially increasing propagation speed (0.5 m/s in small nerves; 130 m/s in myelinated fibers of 20 μm).

The conduction velocity in *myelinated* nerves increases roughly in proportion to the diameter of the nerve e.g. a two-fold increase in diameter means a two-fold increase in conduction velocity.

Myelin reduces space requirements and makes nerves function faster. How? Rather than each small segment of the neuron undergoing depolarization-repolarization, this occurs only at the small junctions (nodes of Ranvier) between the myelin segments. Thus, the electrical impulse leaps down the axon from node to node.

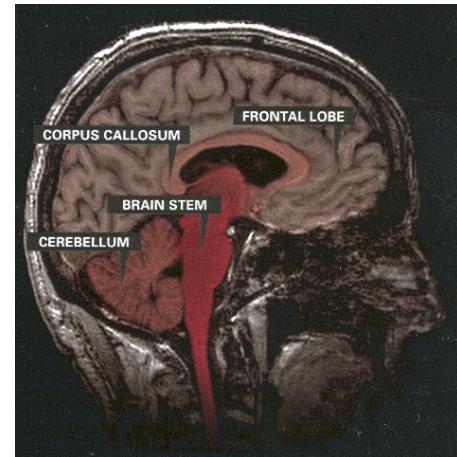
Once the electrical impulse has reached the terminus (bouton) of the axon, the ionic changes stimulate the release of neurotransmitters from storage vesicles to diffuse (by exocytosis) out of the bouton membrane and across the synaptic gap to bind to a receptor on the surface of the next cell (neuron, muscle, etc.) to initiate a new electrical stimulus.

7.1.4. Oxygen Depletion

Although the brain accounts for only 2 % of body weight, it consumes 15 % of the oxygen. When oxygen levels in the blood become too low, neurotransmitter release in the frontal lobe is affected, leading to impaired memory and reasoning. Blood vessels in the brain allow plasma leakage into many

areas of the brain (pictured here), leading to cerebral edema: a staggering gait, coma and death.

F7.5. Oxygen depletion in the brain (red regions). Anoxia in the frontal lobe causes impaired memory and reasoning. Plasma leakage in the corpus callosum creates internal pressure. When the cerebellum is affected, a staggering gait results. Coma and death result from further stress on the brain stem.



7.2. Classification of Neurotoxicants

Neurotoxicants are legion and come from all categories, as can be seen from T7.6. Solvent neurotoxicity is commonly reported in the literature.

Neurologists classify neurotoxicants according to their own functional nomenclature, as follows.

Blocking agents: botulinum (bacterium) toxin prevents release of acetylcholine, tetrodotoxin (puffer fish) blocks the sodium channels.

Depolarizing agents: batrachotoxin (frog skin) destroys the sodium gradient, DDT and pyrethrins (chrysanthemum flower) increase sodium permeability.

Stimulants: strychnine (from tree seeds, rat poison) reduces the effect of the inhibitor glycine, picrotoxin antagonizes the inhibitor GABA, caffeine inhibits the breakdown of cAMP (a second cell messenger).

Depressants: carbon tetrachloride, alcohol, barbiturates (mechanisms less well known).

Receptor antagonists: atropine (nightshade plant) competitively bind cholinergic receptors, propanolol binds receptors of adrenalin and norepinephrine.

Anticholinesterase agents: organophosphate and carbamate insecticides increase stimulation of cholinergic nerves by impairing the destruction of acetylcholine in the synapse.

Neuro-muscular blockers: curare blocks acetylcholine action, succinylcholine prevents membrane repolarization.

T7.6. A list of common industrial neurotoxicants.

Morbidity and Mortality Weekly Report, Vol. 35, No.8, p. 114, Feb. 26, 1986.

Acetyl ethyl tetramethyl tetralin	Acetyl pyridine	Acrylamide
Adiponitrile	Alkyl phosphates	Aluminium
Aniline	Arsenic, inorganic	Arsine
Aryl phosphates	Azide	Barium
Benzene	Boron	p-Bromophenyl acetylureas
Cadmium	Carbon disulfide	Carbon monoxide
Carbon tetrachloride	Chlordane	Chlordecone
Chloroprene	Cobalt	Cuprizone
Cyanide	2,4-Dichlorophenoxy acetic acid [2,4-D]	Dichlorodiphenyl trichloroethane [DDT]
Diethyl ether	Diisopropyl fluorophosphate [DFP]	Dimethyl sulphate
Ethylene dichloride	Hexachlorophene	n-Hexane
Hydroquinone	Lead	Lead, tetrathyl
Leptophos	Malonitrile	Manganese
Mercury	Methanol	Methyl bromide
Methyl chloride	Methyl n-butyl ketone	Nickel [carbonyl]
Nitrogen trichloride	Organochlorine insecticides	Organophosphate esters
Organotins (triethyltin)	Paraquat	Phenol
Phenyl mercury	Phthalate esters	Polybrominated biphenyls [PBBs]
Selenium	Styrene	Sulfur dioxide
Tetrachlorobiphenyl	Thallium	Toluene
Trichloroethylene	Triorthocresylphosphate [TOCP]	Vanadium, inorganic salt
Zinc	Zinc pyridinethione	

7.3. Sites of Attack

F7.3A points to the four vulnerable sites for neuronal damage by chemicals.

1. the dendritic end of the neuron, these fine, filamentous (hair-like) extensions having no protective myelination and only a thin membrane,
2. the cell body of the neuron is usually exposed, with very little protection other than the normal, thin, cell membrane,
3. the axon, either unmyelinated or myelinated, vulnerable because of its length and the necessity of transporting nutrients from the cell body to the bouton and the metabolic products back to the cell body. Attack can occur at either the axon or the myelin,
4. at the bouton itself, with an effect on the membrane or on the neurotransmitters released.

With a more *functional* view, the types of attack could be as follows:

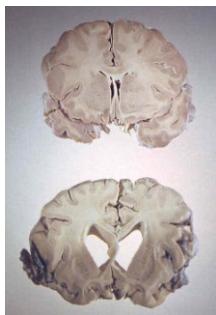
1. on protein synthesis,
2. **axonopathy**, electrical impulse propagation along axons and axonal transport of nutrients using the microtubular system. Axon degeneration usually starts at the end of the axon and grows proximally,
3. on neurotransmitter secretion, action and turnover,
4. **myelinopathy**, decay of myelin sheath. The classic natural disease is multiple sclerosis, which results in pain, loss of coordination and vision. Loss of myelin (death of the Schwann cells) affects nerve conduction velocity. For example, heavy use of organic solvents can lead to walking problems. Also, the bacterium that causes leprosy directly damages myelin sheathing¹⁵.

Agents: Cyanate, lead, chronic cyanide, carbon monoxide. Remember Resdan shampoo, featuring *hexachlorophene*? Hexachlorophene is a good bacterio-static anti-biotic

against *Staphylococcus Aureus*. Unfortunately, it also produces myelinopathy. Fortunately, it does not penetrate the skin well, and its toxicity was detected in burn victims.

- 5. Blood-brain barrier alterations (glue sniffing, microwaves)
- 6. Astrocyte alterations (brain's cleaners disabled)
- 7. Peripheral nerve damage: acrylamide, arsenic, methanol. Some culprits, such as n-Hexane, have been banned.

Polyneuropathy can involve more than one mechanism, in the same way that more than one organ can be intoxicated by a single agent. n-Hexane produces polyneuropathy.



F7.6a. At the anatomical level, neuropathy can be peripheral or central. A graphic loss of central neurons can be seen in Alzheimer's disease (bottom), where the temporal lobes formerly filled with neurons providing memory and language are now empty ventricles.
A normal brain is on top.

7.4. Reversible/Irreversible Effects

In mammals, a severed nerve in an arm or leg regrows and re-established functional connections. A similar injury in the spinal cord or within the brain will not be repaired, resulting in permanent disability and paralysis. Poor regeneration in the central nervous system has been attributed to proteins embedded in brain myelin (the membranes that wrap each nerve axon), which interact with an inhibitory receptor on neurons called NgR.

Many *acute exposures* with low molecular weight compounds produce **reversible** effects in nervous tissue.

Volatile organic solvents affect permeability of cell membranes, so that membrane potentials are altered. CNS depression and anesthetic effects result. Typical agents are nitrous oxide, halothane, methoxyflurane, chloroform, ketones and aldehydes, and solvents found in quick-drying glues. However, *repeated use* of any of these may result in damage so extensive that repair and regeneration mechanisms cannot cope.

According to some sources, the average adult loses 85,000 brain cells per day, but only regenerates 50. There are about 100 billions cells in the adult brain. This limited regeneration is an idiosyncrasy: adult canaries regenerate brain cells quite readily²⁴.

New brain cells are produced by exercise, estrogen, stimulating environments, high social status, electroconvulsive therapy²², stroke and other injuries and antidepressants. It appears that cannabinoids promote neurogenesis, and produce anxiolytic and antidepressant effects.¹⁶ This is very different from other drugs of abuse, which suppress neurogenesis (nicotine, heroin, cocaine).

Neurons involved in olfaction and memory are produced into adulthood. Fewer cells are produced as a consequence of aging, spikes in stress hormones, sleep deprivation, barren environments and Ritalin.

Developing brains may be more susceptible to chemicals. Common anesthetics administered to children (midazolam, nitrous oxide and isoflurane) induce apoptosis in baby rat models, which leads to lasting memory and learning deficits. Exposed rats took longer to learn and tended to forget quickly, probably because of observed cell deaths in the hippocampus. In humans, delicate periods for brain development cover from the third trimester of pregnancy to 2-3 years after birth¹¹.

Anoxia can result from these agents, as well as excessive stimulation with excitatory transmitters, leading to "organic

brain syndrome or disease". Mixtures tend to be far more toxic, due to inter-chemical potentiation.

For example, toluene potentiates the toxicity of all other solvents, while methyl ethyl ketone potentiates the toxicity of n-hexane and methyl n-butylketone.

In *chronic exposures*, myelinopathies, axonopathies or destruction of cell populations (Parkinson's) are more often seen. These effects are **irreversible**...

- ✚ Unborn children transplacentally exposed to **methyl-mercury** poisoning exhibit symptoms of cerebral palsy at birth. Histology of the brain tissue after their death shows absence of myelin and astrocytes ("infantile" brains without normal maturation).
- ✚ There is an inverse relation between a mother's milk level of PCBs (**polychlorinated biphenyls**) and the IQ of children. When at least 1.25 µg per gram of milk is present, the IQ averages 6.2 points lower. Effects are developmental delays, distractability, short-term memory and planning skills impairment. PCBs were used as insulation for transformers and now taint most soils and water. These effects in the children are permanent.
- ✚ Utility workers used to wash their hands in PCBs, secure in the knowledge that PCBs were "only dangerous when burnt" (furanes are then generated). Polybrominated diphenyl ethers (PBDEs[⊕]), used as a flame retardant in foams, have a toxicity profile resembling PCBs¹⁴ (birth defects, thyroid imbalance, neurological damage).
- ✚ Permanent damage is also produced by DDT and manganese. Children drinking manganese-tainted water at levels between 0.2 and 1 mg/l showed an inverse

[⊕] Two PBDEs have been phased out of production.

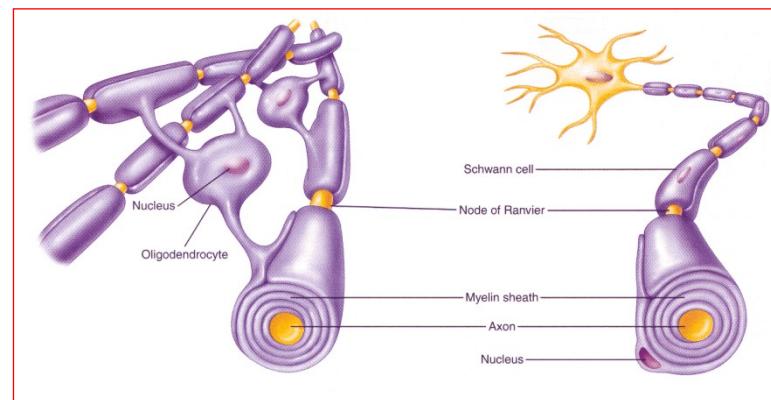
correlation between Mn and IQ scores¹⁷. Fortunately, Mn contamination of water at dangerous levels gives it a nasty smell.

The difficulty of assessing clinically neurological disturbances is illustrated by the Gulf War syndrome, where marginal exposures to toxicants produced headaches, forgetfulness and mood disorders. 60 % of these people show reduced blood flow in the cortex. There is great conflict and discussion over the existence of the syndrome.

7.5. Myelin and Myelinopathy

There are differences between myelination in the peripheral (both sensory and motor) and central nervous systems. This is important to understand the distinction between central and peripheral myelinopathies induced by chemicals¹.

The myelin⁸ sheath in peripheral nerves is from a specific cell, the *Schwann cell*, which wraps itself around a portion of the neuronal axon in much the same fashion as a window roller blind. The nucleus of the Schwann cell resides on the outer surface of the rolled myelin layers. A number of Schwann cells, side by side, provide the myelin for one neuron.



F7.7. Myelin sheath anatomy in the peripheral and central nervous systems. Human Physiology, Benjamin Cummings, 2002.

The junction between the edges of two Schwann cell myelin projections form the nodes of Ranvier, the point of neuronal depolarization, a thin "tunnel" into the membrane of the neuron. Damage to a single Schwann cell can result in its death, with a gap of nonmyelinated axon surrounded by debris. The neuron conducts pulses rapidly down to the point of the damage, but pulses slow down over the defect, resulting in the disruption of smooth conduction.

In contrast, in the CNS, the role of the Schwann cell is carried out by a specialized cell, the *oligodendroglial cell* which can produce a plethora of sheaths around several adjacent axons, rather than just on one axon. Destruction of one oligodendroglial cell results in the changes in a group of neuronal axons, and may result in more than one observed adverse effect. Unlike peripheral myelinopathy which tends to be relatively specific or localized in effect, a centrally located myelinopathy may appear quite *diffuse*. Multiple sclerosis is one such myelinopathy.

7.5.1. Solvent Myelinopathy

The short-term alcohol-like symptoms of solvent neurotoxicity are headache, dizziness, sleepiness, agitation, euphoria and confusion⁹.

The chronic symptoms are premature aging, memory impairment, mild depression and anxiety.

The solvents are typically chlorinated hydrocarbons, alcohols, esters and ketones. They are used for cleaning, degreasing, thinning, stripping and finishing.

The least toxic are alcohols, esters and aliphatic hydrocarbons. Aromatic hydrocarbons and most ketones are highly toxic, and halogenated derivatives of hydrocarbons damage the CNS, liver and kidneys. Benzene is both a neurotoxin and a carcinogen.

Among chronically exposed workers, the disease is common but difficult to recognize because of highly variable, inter-individual responses³. Individual diagnosis is difficult even with the best test batteries, each victim presenting one or a few symptoms^{2,3}. Victims often present at a relatively young age with serious neurological sequelae from excessive exposure to solvents by inhalation, and are forced to retire from their work. Most of the neuro-psychological symptoms appear to be relatively minor, but still involve demyelination in the central nervous system. The effects are fatigue, sleeplessness, headaches, irritability and nausea as well as memory and concentration difficulties.

Problems occur with other organs, such as liver, kidneys, skin, mucous membranes, heart, eyes and reproductive system.

The mechanisms by which solvents induce such effects is unclear and many theories exist. However, many chemicals are capable of nonspecific interactions with neurological membrane phospholipids, causing instabilities and interference with conduction, as well as causing damage to Schwann or oligodendroglial cells and subsequent alterations in myelinated pathways.

7.6. Hexacarbon-Induced Polyneuropathy-Axonopathy

n-hexane and methyl-butylketone cause classical peripheral nervous system effects. They were at one time used in fast-drying glues in the shoe industry and responsible for outbreaks in several countries such as Japan, Italy, Turkey and USA. These two chemicals can be considered together since they share a common toxic metabolite, 2,5-hexanedione⁷.

The associated pathology, first detected in humans, was taken afterwards to animals models in rats and chickens. Scientists

returned to the human situation with recommendations for modification of glue formulations, which produced fewer neurotoxic metabolites.

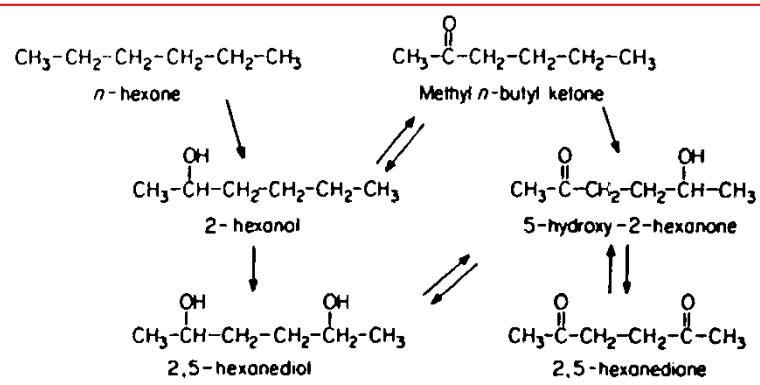
The neuropathy, reported among workers in the shoe industry, was always most severe in the early springtime, following a winter of closed doors and windows (poor ventilation). The signs and symptoms listed in T7.8 are matched morphologically with Ranvier paranodal swelling at the distal end of myelinated axons, with a progression of the swellings toward the proximal portion of the nerve axon. These swellings cause disruption of the microtubule and neurofilament system responsible for the transport of nutrients to the distal part of the axon from the cell body, with eventual death and disintegration of the nerve endings. If recognized early enough, the syndrome is reversible.

T7.8. Symptoms of hexacarbon neuropathy.

- Weight loss
- Gradual onset of distal paresthesia
- Muscle weakness - distal (hands, feet)
- No ataxia or muscle spasticity
- Symmetrical dysfunction (hands, feet)
- Loss of sensation - “stocking and glove”
- Persistent muscular weakness
- Atrophy of musculature

Experiments in animal models revealed that not only were the distal parts of fine nerves of the peripheral nervous system affected, but the distal portions of fine myelinated nerves in the spinal cord (lumbar, sacral) were also damaged, showing much the same morphology. In the paranodal swellings tangles of microtubules and filaments were observed, giving the

appearance of a bird nest. Nutrients are no longer transported to the distal end.



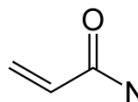
F7.9. Partial metabolic pathway for n-Hexane and methyl n-butyl ketone.

n-Hexane is transformed by two successive hydroxylations and carbonylations (F7.9). The central-peripheral distal axonopathy, so named by neurologists because it affects distal axons of both the central and peripheral nervous systems, is caused by a reactive metabolite, 2,5-hexanedione, found to cyclize during further biotransformation to form a 5-membered ring (imidazole) that interacts with protein structures to disrupt neural organization and cause the tangles. The complete mechanism has not been entirely identified, and interest has declined with the banning of n-hexane and methyl-butylketone and the substitution of other volatile ketones.

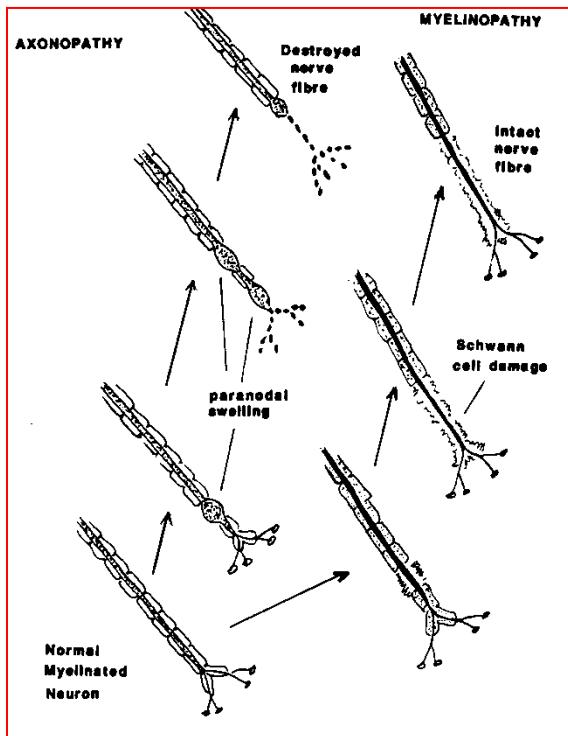
F7.10. Formation of axonopathies and myelinopathies.

Humans take a long time to bio-transform and excrete a single exposure to these agents, peak elimination in the urine occurring between 12 and 48 hr after exposure. The workers were exposed daily to high

concentrations (estimated as high as 2500 ppm, TLV = 50 ppm) throughout the winter months when ventilation in homes and factories was poorest. When such workers were removed from exposure, the condition was not alleviated, but continued to worsen for approximately 14 days, after which recovery began slowly. The condition was only partly reversible. 2,5-hexanedione also attacks the gonads (testicular atrophy).



Acrylamide (shown) and the catalyst DMAPN (di-methyl-amino-propio-nitrile) cause the “stocking-and-glove” central-distal axonopathy by a somewhat similar mechanism.



This is seen in tunnel workers who inject an acrylamide resin using a pressurized lance into soil and rock to create an impervious, water-proof layer to prevent leaking.

7.7. Metals Neurotoxicity

The term "heavy metal" describes a class of agents in the periodic table possessing certain chemical similarities, including a divalent positive ion. Cadmium, cobalt, lead, magnesium, manganese, mercury, nickel, and vanadium are toxicologically important and all are neurotoxic.

7.7.1. Lead

7.7.1.1. Adults

A millennium ago, body concentrations of lead were typically one-hundredth to one-thousandth of post-industrial levels. The average blood lead level today in the US is less than 2 µg/dL. In the early 1970s, blood lead levels up to 40 µg/dL were considered safe. Today, in adults, levels of 30 µg/dL are tolerated. OSHA will permit a worker to return to work at 40 µg/dL, after being medically removed from work because of lead poisoning.

Long-term exposure to low levels of lead may result in the development of learning and behavior problems, cardiovascular and kidney diseases, decreased fertility, hypertension and cancer²⁵. Blood lead levels ranging from as little as 20 to 29 µg/dL are associated with a 39 % increase in mortality from all causes, a 46 % increase in mortality from cardiovascular diseases, and a 68 % increase in mortality due to cancer.

Methyl-mercury is associated with the risk of myocardial infarction, and this partially offsets the protective effects of consuming fish²⁶.

Overexposure to inorganic lead continues among workers in battery manufacturing, mining of lead and zinc ores, and

painting and paper-hanging. In adults, acute lead exposure leads to renal proximal tubular damage, while chronic exposure can cause renal failure, hypertension, hyperuricemia, and gout²⁷. Work-related lead exposure remains a problem, and prevention needs to be strengthened because of lead's low dose toxicity.

7.7.1.2. Children

Lead is especially toxic to the central nervous system, affecting mental development and intelligence in children. Behavioral disorders such as attention deficit disorder have also been attributed to lead exposure²⁸. In children, 20 to 25 mg/100 ml can cause irreversible brain damage²⁹.

Studies of lead in city children initially drove the lowering of acceptable lead concentrations in the blood to 10 µg/dL. But IQ in children further increases as blood levels are lowered to 5 µg/dL⁶. Within the range 1-10 µg/dL, for each µg/dL increase in blood lead level in children, there is a 0.82 point IQ deficit. Above 10 µg/dL, each µg/dL increase only corresponds to a

Acute lead poisoning kills children in the state of Zamfara, Nigeria

A total of 163 children out of 355 cases from several remote villages have died of lead poisoning. The state of Zamfara retained a Chinese company to mine gold in ground that also had high concentrations of lead, but villagers attempted to capitalise by illegally digging for the precious metal themselves. The deaths were discovered during the country's annual immunisation programme, when officials realised there were virtually no children in several remote villages. Villagers said the children had died of malaria, but blood tests from local people showed high concentrations of lead, pointing to death from lead encephalopathy. It is likely locals became sick after lead removed during the process of refining gold ore contaminated local water systems.

0.13 point loss. It is probable that any lead in the blood decreases IQ.

2.2 % of children aged 1 to 5 are above 10 µg/dL, 10 % are above 5 µg/dL and 90 % are above 1 µg/dL¹⁰. The best predictor of lifelong IQ outcome is lead level in the blood at 2 years of age. So, be careful of "pica" !

Similar effects on cognitive developments in children have recently been found in relation to Polycyclic Aromatic Hydrocarbons (from combustion of fossil fuels)²¹.

For every 5 µg/dl increase in a child's average blood-lead level (ages 5 and 6), there is a 25 percent increase in the number of violent activities associated with criminal arrests later in life²³.

Lead acts by multiple mechanisms in the central nervous system⁴. In neurodevelopmental toxicity, interference with cell adhesion molecules causes a mis-wiring of the central nervous system during early development, and permanent dysfunction. This is a major concern for young children who absorb lead much more efficiently than adults, and who may come into contact with higher levels than adults.

Another toxic mechanism involves interactions between lead and other essential divalent cations (calcium and zinc), these ions having vital roles in neurotransmission²¹.

Lead can also act on retinal tissue through apoptosis¹². Early prenatal exposure to lead causes kids to have retinal deficits up to 10 years later.

7.7.2. Methyl-mercury

Mercury has no known metabolic function, and is the most toxic non-radioactive heavy metal. It affects nerves, muscles and all organs. In inorganic form, it is most injurious to the kidneys, while its organic form is very neurotoxic.

Mercury promotes the production of free radicals, and may bind selenium, which cannot then serve as a cofactor for glutathione peroxidase. Mercury may inactivate the antioxidant properties of glutathione, catalase, and superoxide dismutase. Mercury intoxication has been also known as *Minamata disease*, the name of a city in Japan where the offspring of mothers who consumed fish poisoned by local industrial discharges were victims of mental retardation and absence of limbs.

Metallic mercury is poorly absorbed from the digestive system, but mercury as a vapor (broken thermometer) can cross the blood-brain barrier and accumulate in the central nervous system as well as in kidneys, lungs and fatty tissues. It causes dysfunction, acute and chronic inflammation and results in a large variety of symptoms. Mercury-based dental fillings were a bad idea; the problems of toxicity were pointed out as early as 1840. Such fillings are unacceptable today, in view of alternatives.

Inorganic mercury as calomel (mercurous chloride) was used for centuries by physicians, until it was realized that it did much more harm than good. Recently, it was possible to purchase in Mexico a face cream named “Crema de Belleza”, which had calomel as an ingredient. Urine levels found in users and their partners were up to 20 µg/dl. Only 10 % of inorganic mercury is absorbed by mouth, but it affects the digestive system at all levels. The salts also damage the kidneys, leading to reduced urine flow and possible need for dialysis. The familiar antiseptic mercurochrome (a disodium salt of dibromo-hydroxy mercuri-fluorescein) has caused death in children when applied to large burns.

Organic mercury can be 90 % absorbed by the digestive system (methyl mercury). Organic forms of mercury easily travel through organic tissues and accumulate in brain, liver,

kidney, blood, skin, hair and breast milk. The body only excretes 1 % of its organic mercury load every day, and prognosis for this intoxication is very poor.

In the brain, methyl mercury inhibits acetylcholine synthesis, resulting in fatigue, memory loss, mood changes, tremors, pallor, weakness and loss of vision or taste. The symptoms are very similar to those of multiple sclerosis.

This toxicologist, Karen E. Wetterhahn of Dartmouth College, used dimethyl mercury, protecting herself with latex gloves. She spilled a tiny amount on her gloves in August 1996, became ill within a few months, bumping into doors and slurring words, slipped into a coma and died less than a year later. Dimethyl mercury penetrates disposable latex gloves in 15 seconds or less, is volatile and very lipid soluble.

As a result of this incident, MSDS sheets were updated.



7.8. Perfluoro compounds

Some chemicals, such as perfluoro-octanesulfonic acid (PFOS) and perfluoro-octanoic acid (PFOA), used in non-stick fry pans, have been shown to adversely affect behavior of mice¹⁹. The probably carcinogenic compounds have also been shown to act as estrogen mimics²⁰.

7.9. Parkinson's

The motor manifestations of Parkinson's disease are related to a lack of the neurotransmitter dopamine in the brain.

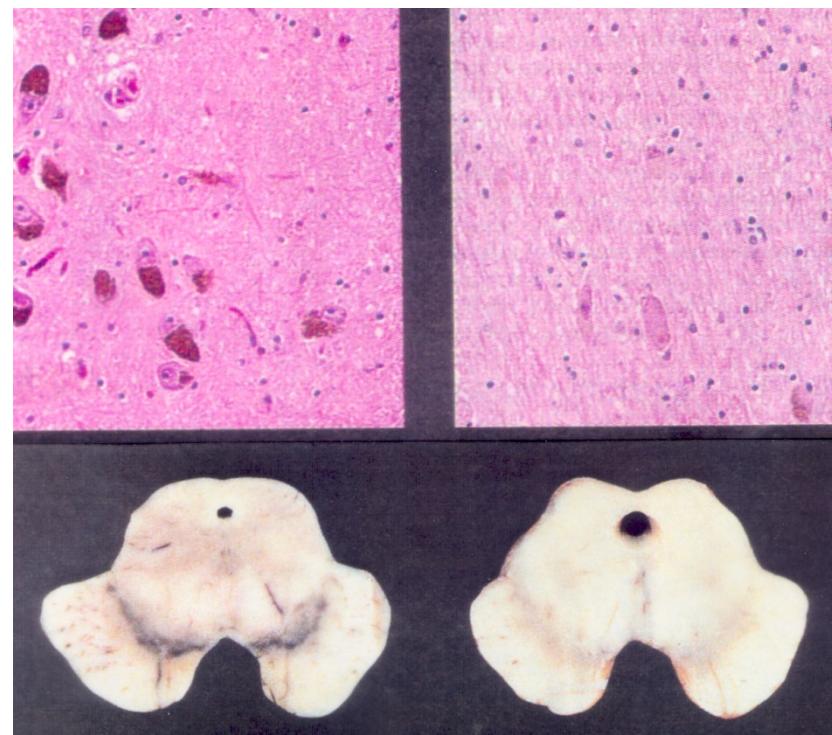
Within the brain, the *pars compacta* (F7.11) of the *substantia nigra* (purple in F7.12) loses neurons which normally provide dopamine signals to the *striatum* (blue). The *striatum* signals control motion in the brain's cortical regions.

The dark brown pigments at top left in F7.11 correspond to dopamine-producing neurons which oxidize dopamine to the pigment neuromelanin. The normal brain at left shows more dopamine both in the histology (dark brown cells) and in the anatomical section (black pigment) than the Parkinson's brain at right.

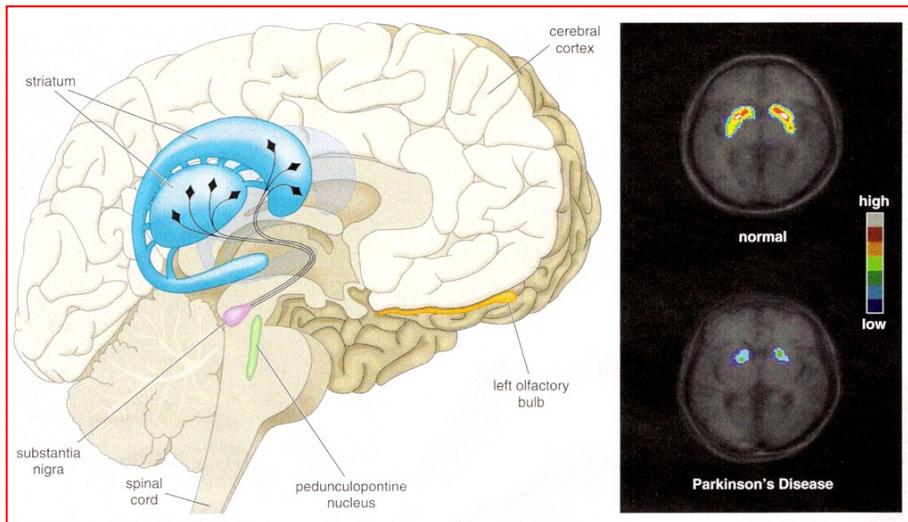
An effective therapy for the motor symptoms is the administration of *levodopa* which can cross the blood-brain barrier and act as a precursor of dopamine.

Although lesions in the dopaminergic system have been prominent in Parkinson's research, this brain disease is not restricted to the *substantia nigra*. Other areas of the brain, the spinal cord and the peripheral nervous system are affected in Parkinson's, as are serotonin, norepinephrine and acetylcholine neurotransmission. Postmortem studies suggest that the brain stem is first affected, then the *substantia nigra*-*striatum*-midbrain, and finally the cerebral cortex.

One strange feature is that in the same individual, some neurons are affected while others are spared, which underlines our incomplete understanding of this disease. Early Parkinson's may manifest by a weakened sense of smell. Late Parkinson's results in non-dopaminergic difficulties, such as dementia.

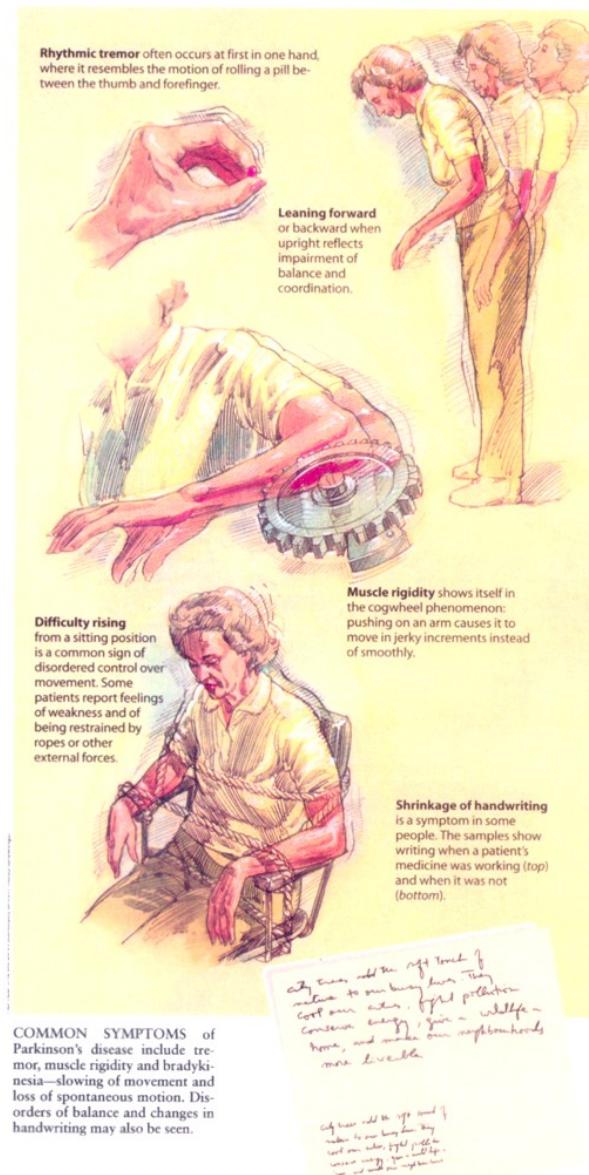


F7.11. Histology of the *pars compacta* in the *substantia nigra*. Section of the top of the brainstem (hole is the central canal) at the level of the *substantia nigra*. *Scientific American*.



F7.12. Neuro-anatomy and PET scan of dopaminergic activity in Parkinson's disease.

American Scientist. Scan by Alain Dagher, McGill MNI.



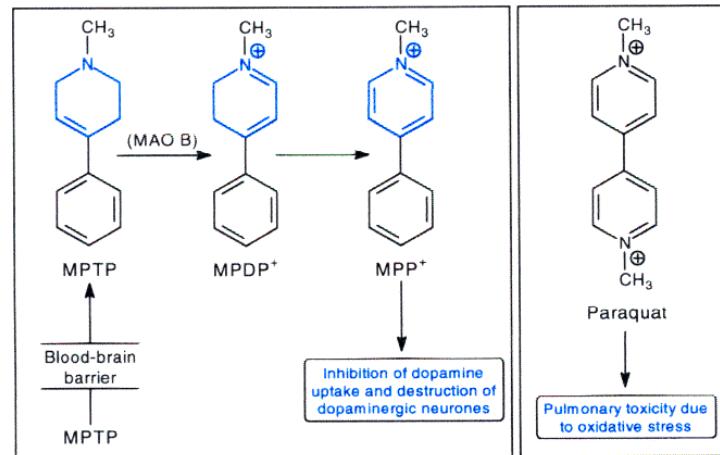
7.13. Common symptoms of Parkinson's. *Scientific American.*



F7.14. The frozen addicts. *Scientific American.*

The exact causes of Parkinson's disease are not known. There are many indications that Parkinson's is related to toxic exposure in the environment, an association long suspected by epidemiologists.

The so-called frozen addicts posed for a picture in 1991 (F7.14) after having received treatment. Nine years earlier, all suddenly became immobile as if they had instantly acquired Parkinson's. They had consumed a narcotic containing the impurity MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) which inhibits mitochondria's electron transport chain. This episode provided a model for the disease. MPTP causes a condition similar to Parkinson's in animals and its molecular structure is related to that of several pesticides (F7.15).



F7.15. MPTP metabolism and action. *Casare & Doull.*

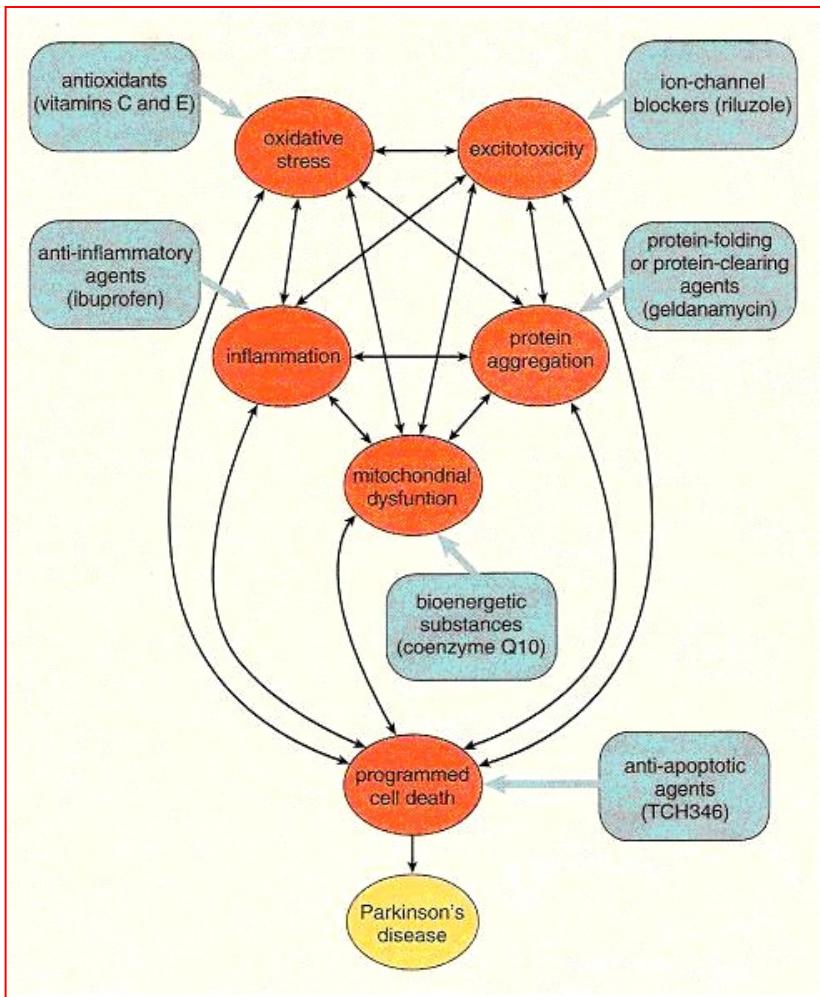
Older people in Guam and in Guadeloupe are victims of a disease very similar to Parkinson's. The disease has been recently linked to eating sago palm (Guam) and soursop, custard apple and pomme canelle (Guadeloupe).

The fruits contain alkaloids (*alkaline-like*, loose group of compounds such as curare, atropine, physostigmine, morphine, codeine, cocaine, caffeine, LSD, quinine, serotonin, strychnine, nicotine). The disease does not respond to *levodopa*, contrary to Parkinson's.

Prolonged administration of rotenone, a plant-derived pesticide used in "organic" gardening produces symptoms of Parkinson's in rats⁵.

As in many neurological diseases, it is difficult for clinicians to determine if a drug therapy actually slows (or reverses) the death of brain cells, or just improves brain function, so that it compensates for the loss of cells. Until this distinction can be

made, the various attempts to deal with the disease by preventing the death of neurons may not be productive.

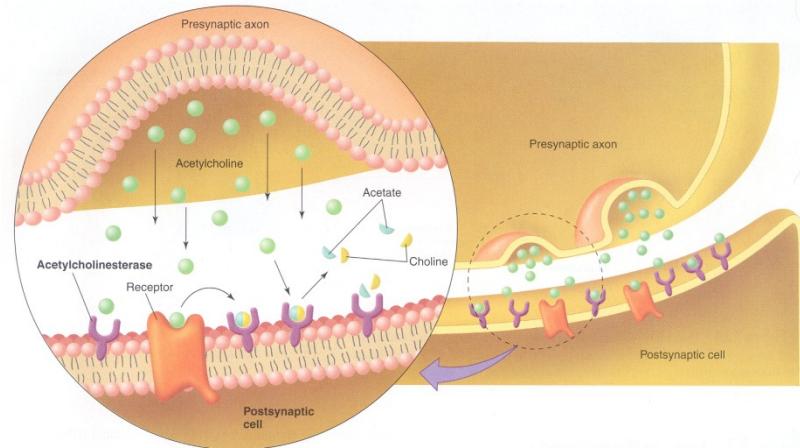


F7.16. Therapeutic thinking for prevention of cell loss in Parkinson's disease (and many chronic ailments). The mechanisms for cell killing are in red, interventions to prevent cell loss are in blue-gray.

7.10. Acetylcholinesterase Inhibition.

There are 2 types of cholinergic receptors: muscarinic (heart rate, bronchoconstriction, arteriole dilatation, secretions of stomach and intestine, salivary and lacrimal glands) and nicotinic (neuro-muscular junction, autonomic ganglia). Acetylcholine will stimulate both of them, but muscarine and nicotine are selective.

The sites of action may be anywhere in the body, but the mechanism is very specific, as depicted in F7.17. Toxicants can have effects on muscarinic and nicotinic receptors and on acetyl-cholinesterase itself (the enzyme responsible for degradation of acetylcholine in the synapse).

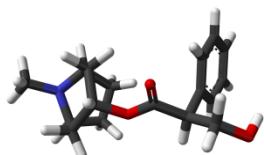


F7.17. Nerve synapse. Toxicants can interfere with neurotransmitter release, stimulation of receptors or the breakdown of acetylcholine by acetylcholinesterase.

Organophosphates and carbamate insecticides inhibit acetylcholinesterase, producing over-stimulation of the cholinergic systems.

For example, organophosphates react with a serine hydroxyl group at the active site of acetylcholinesterase, inactivating the hydrolytic function. This causes an accumulation of acetylcholine esters, leading to cholinergic over-stimulation. In acute or moderate chronic exposures, neuromuscular weakness occurs because of down-regulation of receptor activity. The diaphragm is a red muscle dependent on acetylcholinesterase, therefore breathing can be affected. California has a long-standing formal blood cholinesterase monitoring program for mixers, loaders, and applicators of pesticides. The test looks at two substances, acetylcholinesterase (nerves and red blood cells) and pseudocholinesterase (liver). Normal pseudocholinesterase values range between 8 and 18 units per milliliter.

Intoxication is treatable with *atropine*, which competitively binds the receptors of cholinergic nerves. Atropine (shown), otherwise known as *Atropina Belladonna* has an interesting story. Women in times past would administer themselves atropine



to dilate their pupils before going to court or meeting prospective important lovers. If you are in court, how others perceive you may be your best weapon, so-to-speak putting "your best eyes forward".

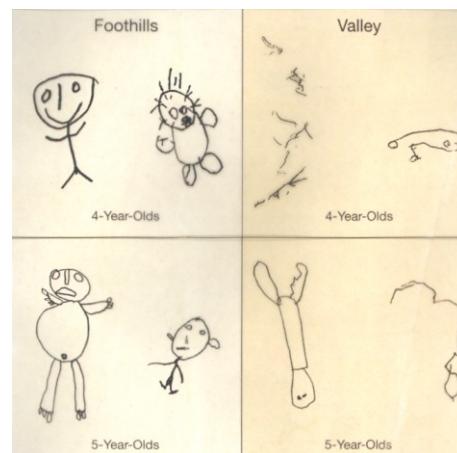
7.11. Consequences of Pesticide Exposures

Neurobehavioral testing of exposed children in Mexico by the University of Arizona showed that exposed children had:

- ✚ less stamina,
- ✚ poorer gross and fine eye-hand coordination,
- ✚ weaker 30-minute recall and
- ✚ impaired drawing ability (F7.18).

It was shown that children are born with detectable concentrations of many pesticides in their blood and are exposed further through breast milk.

Many studies^{30,31,32} have confirmed that children exposed to high pesticide levels in the womb have lower average IQs than other kids. The most heavily exposed children scored an average of 7 points lower on IQ tests compared with children with the lowest pesticide exposures. Since pesticide exposure after birth wasn't linked to lower intelligence scores, this suggests that the harm caused by the chemicals is greatest during early pregnancy, when the brain is developing. Earlier studies have linked organophosphate pesticides with attention deficit hyperactivity disorder (ADHD).



F7.18. Mexican children from "Foothills" (Yaqui Indians) have only one major exposure to pesticides: DDT spraying by the government to control malaria. "Valley" farmers apply pesticides 45 times per crop year and also tend to use household bug sprays daily. These exposures are not significantly different from those in other intensely farmed areas.

7.12. Chemical Warfare Agents

The military are trained to inject themselves with atropine in case of a neurotoxic attack. Organophosphorus warfare agents as well as insecticides can also be countered by certain drugs such as galantamine, a reversible centrally-acting acetylcholinesterase inhibitor.

Galantamine, otherwise used in Alzheimer's treatment, was effective up to 3 hours before and 5 minutes after exposure to the organophosphate¹⁸.

T7.19. Warfare Neurotoxicants.				
Agent	Appearance & odor	Absorption	LD ₅₀ (mg/m ³ .min or mg/kg)	Symptoms
Tabun	Colorless to amber-brown liquid; slightly fruity odor	Skin contact and inhalation	400, inhaled 1000, skin	Runny nose, constricted pupils, tightness in chest, blurred vision, nausea, vomiting, confusion, loss of control of body functions, respiratory paralysis.
Sarin	Colorless liquid; no odor when pure		70, inhaled 1700, skin	
VX	Colorless to amber liquid; no odor when pure		50, inhaled 10, skin	

7.13. Neurotoxicity Testing

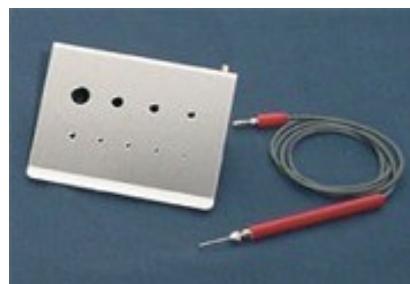
Neurotoxicity testing is complicated by the need to differentiate between CNS and peripheral nerve damage, as well as between reversible and permanent lesions.

Tests in humans can range from sensory-motor (F7.20) to psychological. Batteries of tests give more perspective on neuromorbidity: X-rays, CAT scans, MRI, electromyography, EEG, peripheral nerve conduction velocity and cerebrospinal fluid composition are used. There is some discussion about the definition of the “normal” range appropriate for various results, which is often defined purely by statistics, as opposed to specific thresholds.

In animals models, performance tests such as maze, movement recording and platforms hidden within pools are frequent.

Rats are tested for abnormal posture, closure of eyelids, tremors or convulsions, lacrimation, salivation, gait, mobility, level of arousal, stimulus response, pupil response, righting reflex, forelimb and hindlimb grip strength and foot splay.

Although cells are used to test for neurotoxicity, these models have not made great inroads yet.



F7.20. A device to measure hand tremor.

7.14. Case Study: Chlorinated Hydrocarbon Pesticide

(modified from Williams & Burson)

A physician in Virginia saw a patient who worked in a plant where Kepone (chlordecone) was produced. The patient complained of weakness, nervousness, weight loss, shakiness and difficulty in reading and driving because he could not focus his eyes. The physician thought that the exposure to Kepone might have caused the patient's illness and sent a blood sample to the CDC for a determination of Kepone content. The full chemical name of Kepone is 1,1a,3,3a,4,4a,5b,6-decachlorooctahydro-1,3,4-metheno-2 H-cyclobuta [cd] pentalen-2-one. The CDC used to have a service for physicians in which, on request, would analyze adipose tissues, blood samples, and other biologic samples for content of chlorinated hydrocarbon pesticides. The chemist at CDC who received the sample, not realizing the emergency, thought that the Virginia physician had previously arranged for such an analysis. He therefore accepted the sample, analyzed it, and found very high Kepone levels. He thought his analysis was wrong, so he repeated it three times before he said anything about it to anyone. In the meantime, the patient visited a neurologist at the Medical College of Virginia. The patient's symptoms and signs now included extreme nervousness, tremor, ataxia, skin rash, and an odd rolling of the eyes called opsoclonus. He also had lost weight. He couldn't concentrate even to read a newspaper, and he still had trouble driving.

Ultimately, the high Kepone blood level determined at CDC was reported to the neurologist at the medical college and also to the requesting physician. The toxic effects of Kepone observed in animals include excitability, tremor, weight loss, and, in some cases, testicular atrophy. Kepone is a persistent compound. Once absorbed, Kepone will be stored in body tissues for a significant period of time. It is the only representative of the group of persistent halogenated pesticides that is not predominantly stored in adipose tissue. The ratio between blood and adipose tissue is not as large as for other chlorinated aromatic pesticides and industrial chemicals, and a great deal of Kepone is stored in the liver, because of its greater polarity. After this case was uncovered, we needed some facts about the plant in which the man worked. Several phone calls were made in an attempt to gather information. The neurologist was asked to question the patient about other employees to determine if a larger problem existed. The patient reported that employees at the plant were unable to even drink much coffee during their breaks because they shook so severely they would spill the coffee, or would be unable to fill their cups, an indication that all probably had Kepone-caused tremor.

This was reported to the Virginia state health department; it became apparent that somebody needed to take direct action. The state epidemiologist inspected the plant and closed it, all within two days.

It was a small facility operating out of an old garage. In making Kepone, the firm used a completely open drying operation. During the drying process, dust was spread everywhere. There were no facilities in which the employees could change clothes or wash, and no separate eating facilities.

It was difficult to understand how people could be affected so profoundly-some people were unable to stand up from a chair-and still continue to work without realizing they were being affected by the chemical. In addition to the rash and nervousness noticed in the index case, many people complained of chest pains, but it was not possible to determine a cause for that complaint. It probably was a neurologic effect.

A divorced worker at the plant even seemed to have Kepone-related problems with alimony pay. He would begin to feel sick after working with Kepone, would stay home with no pay. His ex-wife, thinking this an intentional avoidance of work, went to court, and the man would find himself in jail for failure to support her. While in jail, he would recover; and then he would resume work at the Kepone plant, become sick again, and the whole cycle would repeat. Other workers felt that perhaps they were being affected by Kepone, yet they had been told, "This compound doesn't really do anything. It's probably because you drink too much alcohol."

In addition to the workers, residents in the neighborhood were also examined, as well as employees' family members. Sometimes it is difficult to conduct these types of examinations and evaluations objectively because of publicity about the problem. As mentioned, the workers were unable to change clothes at the plant. Workers often carry dust of the chemicals they are working with-particularly persistent compounds, including chlorinated hydrocarbons, lead, and asbestos-home on their clothes and in their cars, thus exposing their families. Many family members of the employees had Kepone blood levels. They were lower than those of the plant employees, but they were higher than what might be expected in the general

population. The Kepone produced at this plant was manufactured for export to other countries. Kepone is not registered for use in the United States, so normally Kepone blood levels are not observed in the general U.S. population. The plant was closed and never reopened. Many political problems remained after the closure, however. The James River was found to be contaminated with Kepone and was closed to fishing for a while. The state of Virginia is still struggling with the problems resulting from this episode of contamination.

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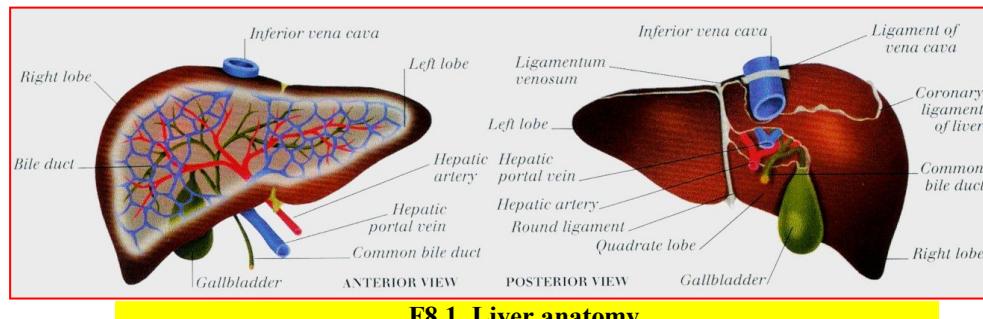
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Hepatotoxicity

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8. Hepatotoxicity



F8.1. Liver anatomy.

The Roman anatomist Galen (200 AD) made the liver the principal organ of the human body, arguing in his text “On the Usefulness of the Parts of the Body” that it emerged first in the formation of a fetus.

Up to 25% of the hepatocytes are binucleate and another 50% are polyploid, reflecting a *need for redundancy* in this vital organ. The liver is a site where *apoptosis* occurs normally, sometimes obviously associated with toxic exposure[◊].

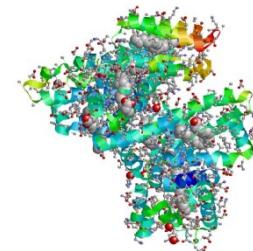
After acute toxic exposure one finds: lipid accumulation in hepatocytes, necrosis or hepatobiliary dysfunction. After chronic exposures, one sees cirrhotic-like or neoplastic changes. Some forms of liver injury are reversible, while others are not.

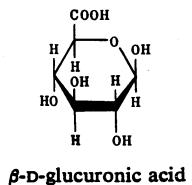
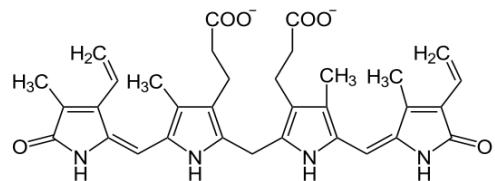
[◊] In rodents, but not in humans, the sedative phenobarbital inhibits apoptosis, leading to considerable liver enlargement.

8.1. Roles of the Liver

The largest gland, the liver performs over 500 known functions....

- 1) Metabolism of **foods** (fats, carbohydrates, amino acids and proteins) and most amino acid interconversions and catabolism, bile secretion to enhance the uptake of dietary lipids and vitamins, urea formation (excretion of nitrogen), storing vitamins A and D.
- 2) It stabilizes the **glucose level** by taking up and storing glucose as glycogen, breaking it down back to glucose when needed, as well as forming glucose from non-carbohydrate sources such as amino acids. Hypoglycemia occurs only late in the course of severe liver disease, because the liver has a large functional reserve, maintaining its function with only 20% of the liver functioning.
- 3) Synthesis of proteins: secretion of plasma **albumin** (a carrier for drugs and hydrophobic compounds such as unconjugated bilirubin, shown), most of the **globulins** (coagulation Factors II, V, VII, VIII, IX, X) other than gamma globulins. Most cholesterol synthesis takes place in the liver.
- 4) Detoxification (turning fat-soluble poisons into water-soluble wastes that can be eliminated through the kidneys) and excretory bile formation, the major product of cholesterol catabolism.
- 5) It removes red blood cells and warms the blood.





For example, hemoglobin transformed to bilirubin is conjugated with glucuronic acid (both shown) in the liver, and the xenobiotics Hg and Pb are excreted in the bile...

Glucuronic acid also conjugates excess estrogens, the resulting products exiting unchanged through the kidneys. Cats are vulnerable to many toxicants because of their limited ability to form glucuronide conjugates.

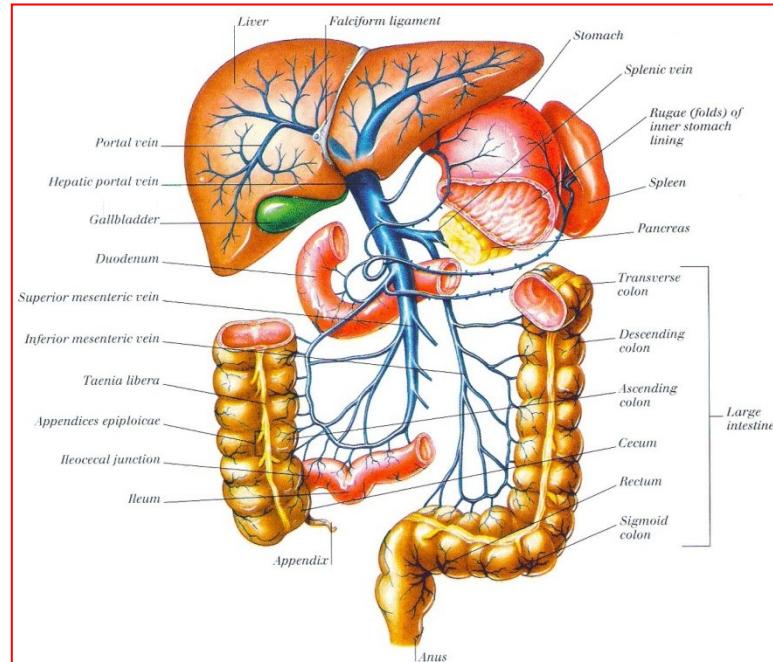
8.2. Anatomy

The liver is the source of the veins and the principal instrument of sanguification, Galen observed. He was not far off the mark, given the organ's position in the circulatory system (F8.2) and its role in intermediary metabolism and biotransformation of xenobiotics.

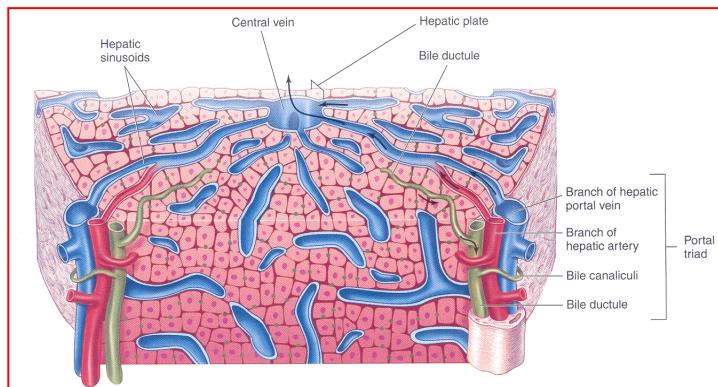
Note the blue portal vein in the center of the diagram, and how blood flows from the various components of the digestive system into the portal vein. Blood output is into the *vena cava*. Some 70% of the liver's blood supply is venous, coming from the mesenteric plexus via the portal vein, while 30% is arterial, from the hepatic artery.

8.2.1. First-Pass

The First Pass effect refers to the influx of toxicants through the portal vein (F8.2), particularly from the digestive system and to the high level of bio-transformation in the liver.

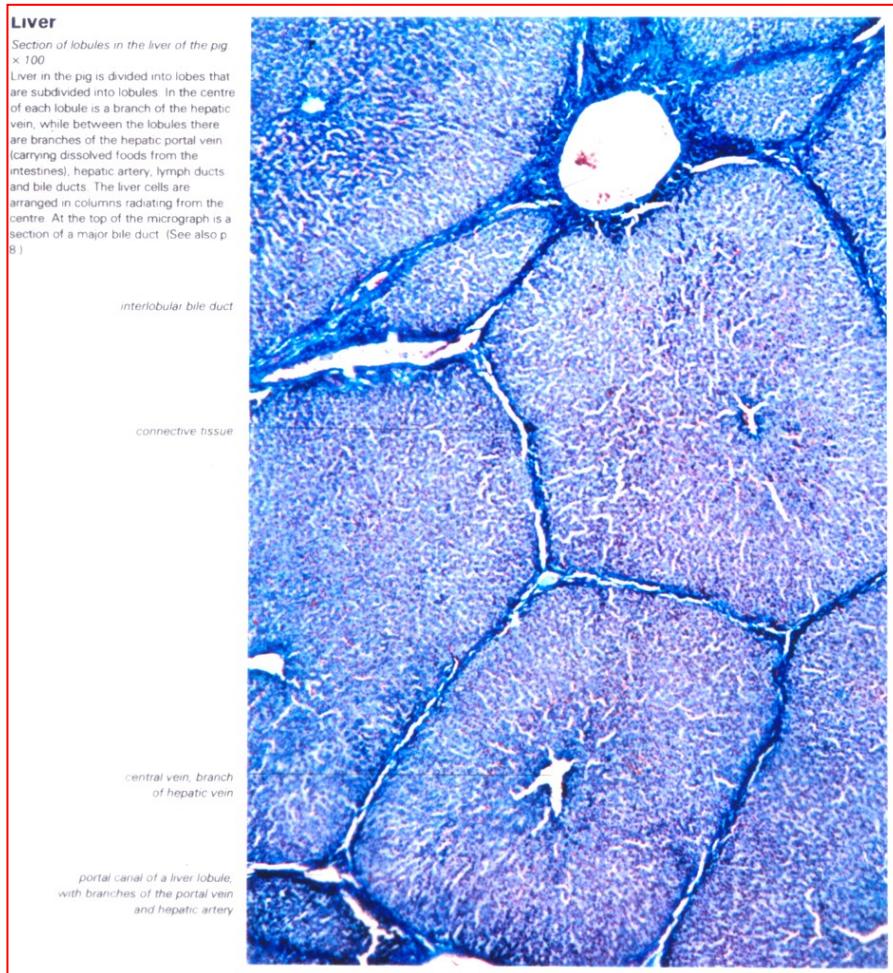


F8.2. Liver's position in the circulation. Dorling Kindersley, 1998.



F8.3. Section of a hepatic lobule. Lobules are 1-2 mm in diameter, visible to the naked eye. Fox, 2004.

On entering the liver, the blood vessels break up into a dense capillary bed, the blood pressure decreases sharply and there is slow perfusion of the blood through the *sinusoids*, permitting



F8.4. Section of lobules in the pig, x 100.
At this magnification, individual cells are barely discernible.
Biological structures.

the bordering cells to extract chemicals from the bloodstream by passive diffusion (F8.3). The functional unit of the liver, the *hepatic lobule*, is a radial array centered on the *central vein*. Branches of three conduits converge towards the center: portal vein, hepatic artery and bile duct. Blood flows through *sinusoids*, while *canaliculari* channel bile. The blood of sinusoids flows towards the center, the bile in the canaliculari away from the center, towards a branch of the bile duct. The sinusoidal lining is fenestrated, allowing chemicals easy access to the hepatocytes, which make up the bulk of the organ. There are at least 6 different types of liver cells: Hepatocyte, Biliary Epithelial, Endothelial, Kupffer, Ito and Pit.

Endothelial cells differ from the vascular endothelium elsewhere in the body in that they lack a basement membrane and contain numerous fenestrae that permit hepatocytes to have ready access to nutrients and macromolecules in plasma. Endothelial cells are also responsible for endocytosis of molecules and particles, and play a role in lipoprotein metabolism.

Spindle-shaped *Kupffer's cells* are tissue macrophages. They form an important part of the body's reticuloendothelial system. Their major functions include phagocytosis of foreign particles, removal of endotoxins and other noxious substances, and modulation of the immune response through the release of mediators and cytotoxic agents.

Peri-sinusoidal fat-storing cells (*Ito cells*) store vitamin A. They transform into fibroblasts[⊕] in response to hepatic injury, contributing to hepatic fibrosis.

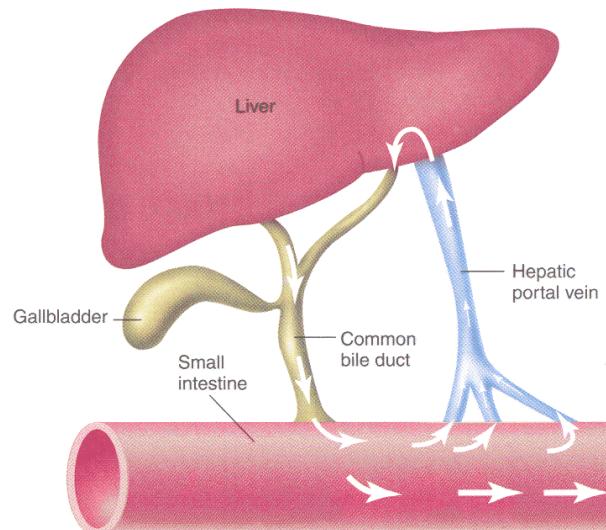
[⊕] Fibroblasts secrete collagen fibers and provide mechanical tension to close wounds.

Pit cells, the least common sinusoidal lining cells, are large, granular lymphocytes, which function as natural killer cells. The extracellular matrix of the liver includes its reticulin framework and several molecular forms of collagen, laminin, fibronectin and other extracellular glycoproteins.

The blood in the liver has a low oxygen content, probably to reduce oxidation reactions. The histologic appearance of the liver is shown in F8.4.

8.3. Physiology

8.3.1. Entero-Hepatic Loop



F8.5. Entero-Hepatic loop.

Compounds that are metabolized (or not) and excreted into the bile duct may be re-absorbed from the small intestine before excretion, resulting in increased toxic exposure.

Phenolphthalein (the active ingredient in Ex-Lax) is an effective laxative because of its persistence through the entero-hepatic loop. After Phase II metabolism in the liver and excretion in the bile, intestinal flora releases it in native form, allowing re-absorption.

Hg is excreted in the bile. If in the form of methyl mercury, it is reabsorbed from the biliary tract, contributing to its long half-life.

However, the increased exposure to toxicants due to the entero-hepatic loop may be a side-show. Up to 95% of bile is reabsorbed through the entero-hepatic loop, and bile plays a large role in the regenerative ability of the liver. This regeneration is tied to the stimulation by the bile of the FXR gene and protein, which acts as a bile sensor. Rats fed bile components in their chow have livers 30% larger within a week. The entero-hepatic loop may support a natural cycle that enhances regeneration in the liver⁷. It is the only internal human organ known to naturally regenerate, from just 25% of its tissue.

8.3.2. Liver Enzyme Distribution

The level of enzymes (F8.6) is not constant throughout the lobule. There is a gradation of activity from portal *input* to vein *output*.

Phase I activities *increase* 5-fold from the portal input to the central vein output.

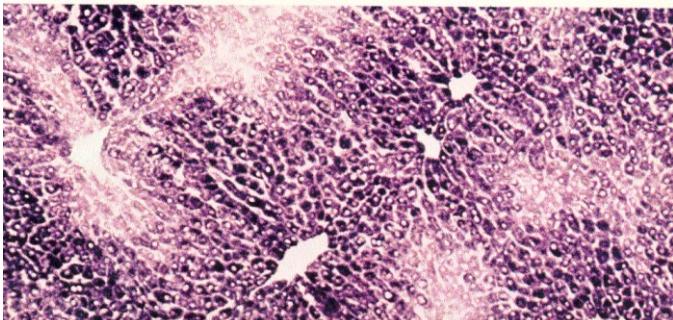
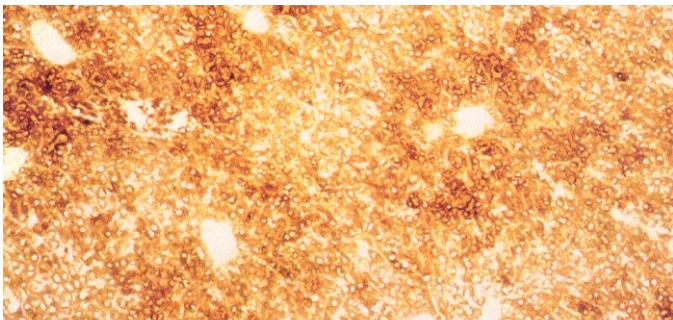
Phase II conjugating enzymes levels (such as glutathione transferase and glucuronyltransferase) *decrease* approximately 4-fold from the portal input to the central vein output.

What does this organization signify when considering toxic reactions in the liver, specifically in avoiding irreversible damage to hepatocytes?

It is probably more efficient for liver cells to specialize in a specific aspect of bio-synthesis, thereby creating two cell populations (Phase I and Phase II).

Since Phase I reactive intermediates are particularly toxic, it is also probably better to provide less enzyme at the portal input, where the highest toxicant concentration is expected. This spreads out Phase I biotransformation and toxic stress more uniformly over the lobule.

Phase II enzymes, less likely to increase toxic stress, may be spread according to the increased availability of bio-synthetic



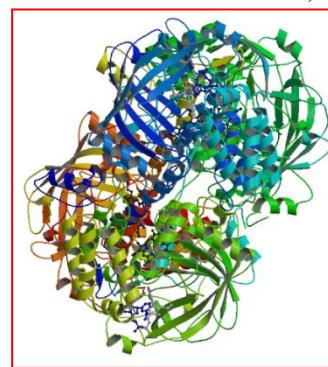
F8.6. Distribution of NADH-oxido-reductase (Phase I, top) and ATPase (bottom) in lobules. X 50. Biological Structures.

machinery upstream, where Phase I metabolism requirements are less. In a high-level insult, there will be a buildup of reactive intermediates in the central vein region where Phase I metabolism is highest. Once enzyme reserves are depleted,

Phase II activity is cast back to a lower basal rate, Phase II being the rate-limiting step in many detoxifications.

These patterns of enzyme expression are not inflexible across species or across time. In male rats, hepatic enzyme distributions alter during aging. Peroxisomes (Phase I) are more active in pericentral than periportal hepatocytes, in contrast to the uniform pattern in young animals. Other age-established lobular gradients were observed in trifunctional enzyme (central > portal) and catalase⁸ (portal > central, molecule shown below). Enzyme levels are significantly

altered in aged animals, perhaps contributing to disturbances in lipid metabolism.



A chemical toxic to the liver may be more toxic when ingested or injected intra-peritoneally than when absorbed through lungs or skin, because disposition of the toxicant to other body

compartments will lower the effective concentration to the liver.

8.3.3. Levels of Cytochrome P450

P450s are heme-containing monooxygenases and are divided into two primary groups: *steroidogenic* and *xenobiotic*.

Steroidogenic P450s are found both in prokaryotes and in the mitochondria and smooth endoplasmic reticulum of eukaryotes. They synthesize steroids and other substances

⁸ Genetic induction of supplementary catalase in mitochondria has been shown to increase the life-span of rats by 15%.

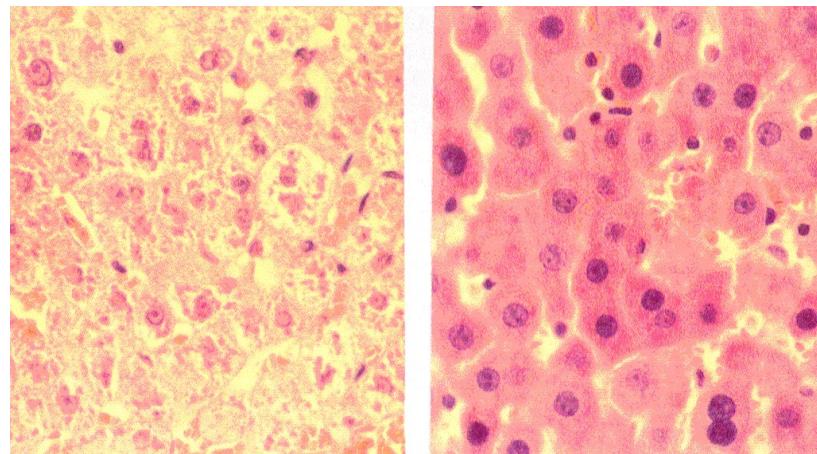
necessary for the maintenance of cell wall integrity and cellular differentiation.

Xenobiotic P450s are found in the smooth endoplasmic reticulum of eukaryotes and metabolize foreign biological substances. There are about 12,000 members across all forms of life, the human ones being encoded by 57 human genes. This group of P450s appears to have evolved from the steroidogenic P450s during the era of plant-animal differentiation, conveying a survival advantage by allowing animals to detoxify the substances that they consumed. These P450s are responsible for most oxidative drug metabolism: CYP3A (~50%) and CYP2D6¹ (~30%) and CYP1A2, 2C9/10, 2C19, and 2E1 which account for approximately equal percentages of the remaining 20%. Relevant P450s have been established for only ~ 20% of marketed medications.

If hepatocytes encounter a moderate or high level of bio-transformable chemicals (say carbon tetrachloride), the cells induce synthesis of additional Phase I and Phase II enzymes. The synthesis is not selective for individual enzymes, rather, a broad spectrum of enzymatic activities are turned on, with the capability of biotransforming a vast array of exogenous chemicals. This is compatible with adaptation to natural toxins which usually come as a group of related compounds, rather than as a single purified molecule. This involves the synthesis of messenger RNA (mRNA) which is used by the cellular rough endoplasmic reticulum (RER) to synthesize proteins (Phase I and II enzymes) which are either incorporated into a subcellular membranous organelle - the smooth endoplasmic reticulum (SER) or released into the

cytoplasm of the cell as soluble enzymes. These changes are easily visible under the microscope (F8.7).

If the cell copes successfully with this defensive mechanism, the levels of these enzymes will slowly decline back to normal. If it does not, the levels of Phase I and II enzymes will remain



F8.7. Appearance of rat liver cells at x 100 in the normal state (left) and after 16 hours of intoxication by ketamine-xylazine (right). Héroux, 1993.

elevated and cytotoxicity may result from the accumulating reactive intermediates. A wide range of compounds are able to induce increased levels of cytochrome P450 enzymes.

Drugs: Aminopyrine, Amphetamine, Barbiturates, Chloral hydrate, Chlordiazepoxide (Librium), Chlorpromazine, Diazepam (Valium), Diphenhydramine, Ethanol, Ethanol pyridione, Glutethimide, Halothane, Hypericin and Hyperforin (St. John's wort), Imipramine, Meprobamate, Morphine, Nicotine, Phenylbutazone, Phenytoin, Promazine, Propoxyphene (Darvon), Steroids, Sulfanilamide, Thalidomide, Trimethadione, Urethane, Zoxazolamine.

¹ CYP2D6 has more than 75 known genetic variants, resulting in poor, intermediate, extensive or ultra-rapid activity.

Industrial chemicals: Alcohols, Aldrin/dieldrin, Chlordane, Chloroform, DDT, DDD, DMSO, Heptachlor, Ketones, Lindane, PCB compounds, Piperonyl butoxide, Pyrethrum, Toxaphene.

Polyaromatic hydrocarbons: Benzo(a)pyrene, Dibenzanthracene, 3- Methylcholanthrene.

Some toxicants are *inhibitors* of biotransformation. They achieve the effect through competitive binding, synthesis inhibition of heme or cytochrome P450, inactivation-destruction of cytochrome P450 or of the endoplasmic reticulum. Examples of such chemicals are carbon tetrachloride, bromobenzene and α -naphthyl isocyanate.



Some toxicants are unexpectedly powerful. A rogue tropical seaweed, *Caulerpa taxifolia*, is presently spreading in the Mediterannean. It produces *caulerpenyne*, a toxicant which inhibits fish's P450 enzyme activity to 25% of baseline and alters metabolite composition. The fish do not need to consume the seaweed, just be in the neighborhood !

In hygiene, it is not uncommon to add together the percentages of the TLVs when multiple exposures occur at a single site. The rough nature of such a procedure is clear if we consider that even if the two chemicals have quite identical dose-responses, they are unlikely to have similar half-lives.

8.4. Pathology

Decreased liver function can result in any number of symptoms including fatigue, allergies and chemical sensitivities, acne or broken blood vessels on the skin, intolerance to fatty foods or alcohol, changes in mood or weight, changes in cholesterol or triglyceride levels, nausea and constipation. Overt liver disease

presents with more obvious symptoms such as yellowing of the skin, liver enlargement, itchy skin, dark urine, pale stool, easy bleeding, blood sugar problems and gallstones.

Liver enlargement is an effect frequently observed in pesticide toxicity studies. Increases in relative liver weight of <10% are generally considered to be non-adverse, while increases of $\geq 10\%$ are interpreted as being potentially adverse.

Liver is highly exposed to toxic damage because of its high rate of biotransformation (T8.8).

If the liver did not metabolize benzene, "its half-life would be 100 years" (P450 2E1). Paradoxically, more benzene is metabolized to toxic species at low doses than at high doses ! Liver cells in culture show a progressive fall in expression of xenobiotics metabolism (1-2 days), and although some human B-lymphoblastoid cell lines can be engineered to stably express human cytochromes P450, it has been very difficult to maintain true toxic metabolism *in vitro*.

T8.8. Metabolism of various microsome fractions.	
Organ or Tissue	Metabolic Activity (% of liver microsomes)
Gut	10
Adrenal Cortex	50-75
Testes	10-20
Spleen	5
Heart	3
Muscle	1
Brain	1
Placenta	1
Skin	1

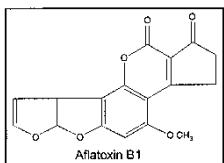
A *cytotoxic agent* may proceed through necrosis or apoptosis. For example, ethanol drives a sequence of cell toxicity, necrosis, fibrosis, resulting in a fatty liver otherwise known as cirrhosis. Cirrhosis often results in jaundice and portal hypertension. The disease may be telomerase-limited. A *cholestatic agent* triggers inflammation of the biliary tree with a decrease in bile flow and excretion.

8.4.1. Anatomical Classification of Liver Injury

T8.9. The Liver Lobule areas endangered by Hepatotoxins.		
Periportal	Midzonal	Centrilobular
Acrolein	Anthropyrimidine	Acetaminophen
Albitocin	Beryllium	Aflatoxin
Allyl alcohol	Carbon tetrachloride	Bromobenzene
Arsenic	Furosemide	Carbon tetrachloride
Iron	Ngaione	Chloroform
Manganese	Paraquat	DDT
Phosphorous		Dinitrobenzene
		Trichloroethylene

8.4.2. Cytological Classification of Liver Injury

T8.10. Hepato-toxicants classified according to their Target.		
Membrane, Endoplasmic Reticulum	Mitochondria	Nucleus
Carbon tetrachloride	Hydrazine	Beryllium
Thioacetamide	Ethionine	Aflatoxin
Phalloidin	Dichloroethylene	Galactosamine
Dimethylnitrosamine	Carbon tetrachloride	Ethionine
Allyl alcohol	Phosphorous	Nitrosamines



Another important pathology of the liver is *hepatocellular carcinoma*, characterized by large malignant tumors throughout the liver (shown) and hepatomegaly. The most potent hepatocarcinogen known is aflatoxin B1, from the *Aspergillus* mould.

8.4.3. Pathological Classification of Liver Injury

T8.11. Hepato-toxicants classified according to mode of action.	
Cytotoxic Agents	Cholestatic Agents
Acetaminophen	Anabolic steroids
Aflatoxin	Arsphenamine
Allyl alcohol	Chlorpromazine
Bromobenzene	Diazepam
Carbon Tetrachloride	Estradiol
Dimethylnitrosamine	Mepazine
Phosphorous	Triiodizadine
Urethane	



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F8.12. Large parts of this liver are invaded by carcinoma nodules (mostly to the left).

8.4.4. Substances promoting Detoxification

Vitamin C is required for Phase 1 detoxification, scavenges free radicals and protects against damage from reactive oxygen species (ROS).

N-acetyl-cysteine (NAC) is a glutathione precursor and protects against ROS damage. Reduced glutathione interacts with toxins such as heavy metals to make them less reactive and thereby less damaging to cells. The oxidized glutathione needs to be replenished. Toxicants such as mercury are removed from the body by direct conjugation with glutathione¹¹. Glutathione can be depleted with increased free radical production during Phase 1 detoxification, resulting in decreased glutathione conjugation in Phase 2 detoxification, and the build up of toxins and heavy metals in the liver.

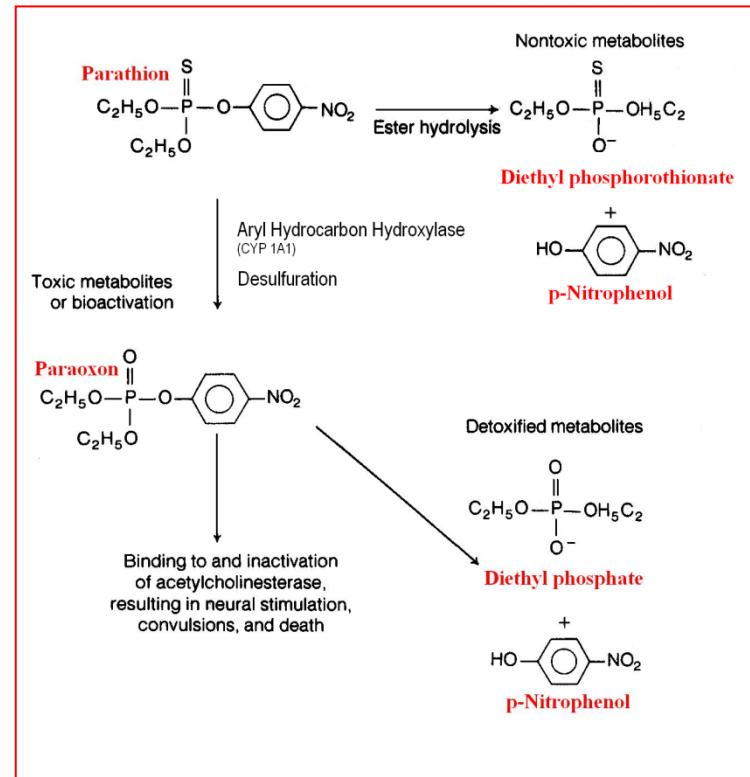
Lipoic acid is a unique antioxidant in that it is both water- and lipid-soluble. Lipoic acid supplementation may be beneficial for the management of liver diseases associated with oxidative stress: alcohol-induced damage, mushroom poisoning, metal toxicity and carbon tetrachloride poisoning¹⁰.

8.5. Action of Toxicants in the Liver

The quest for potency and efficacy in pharmaceuticals leads industry to purify the active ingredients of natural medicines, so that they can then be synthesized in pure form. Pure forms of medicines may reduce toxicity from other ingredients, but may also concentrate metabolic stress on specific pathways. Depending on the toxicity of a therapeutic, it may sometimes be advantageous to use natural extracts, which may induce less toxicity as well as take advantage of an array of molecules with similar action.

8.5.1. Enzymatic Activation of Toxicity

The liver's metabolism can activate toxicity in its attempts at detoxification. We show here three examples in which this occurs.

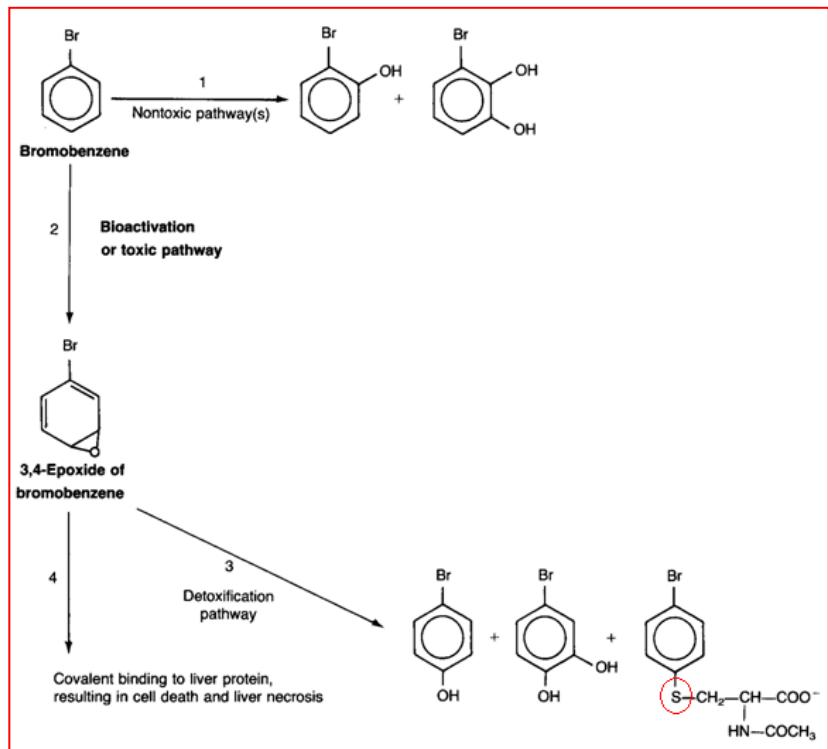


F8.13. Parathion metabolism. *Williams and Burson.*

8.5.1.1. Parathion

Parathion is converted to the toxic form (F8.13), **paraoxon**, by the enzyme AHH (also known as CYP1A1), resulting in local hepatic toxicity.

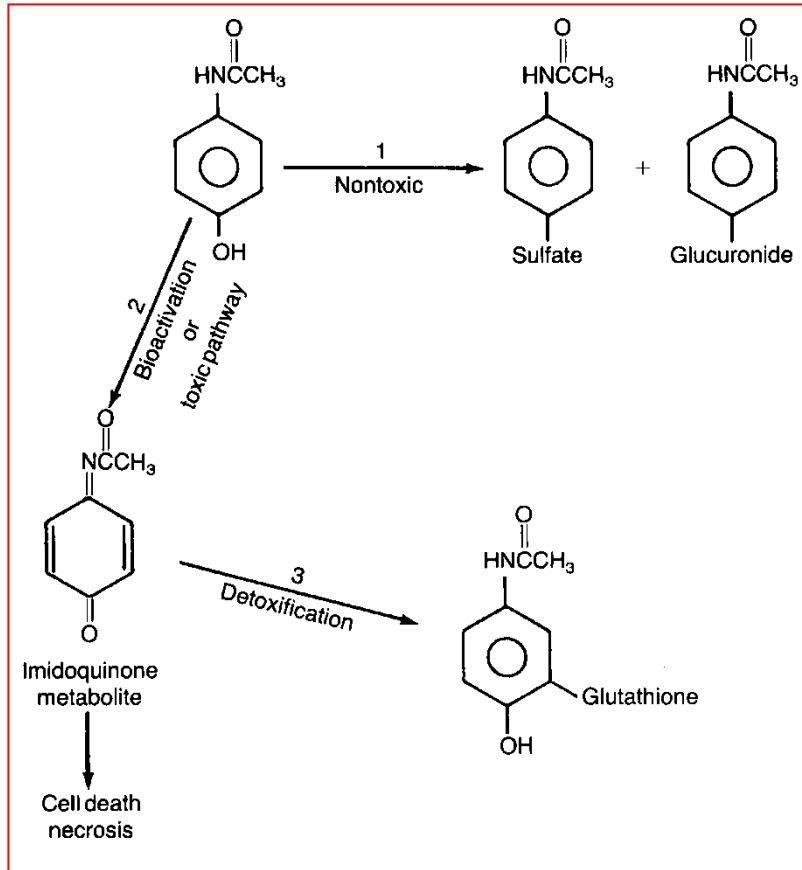
Clinically, attempts are made to remove it from the body by dialysis and charcoal ingestion. Atropine would be administered to counter its debilitating effects on the nervous system.



F8.14. Bromobenzene metabolism. *Williams and Burson.*

8.5.1.2. Bromobenzene

Bromobenzene results in centrilobular necrosis and is also a powerful nephrotoxicant. It is metabolically detoxified using glutathione's sulfur bond.



F8.15. Acetaminophen metabolism. *Williams and Burson.*

8.5.1.1. Acetaminophen

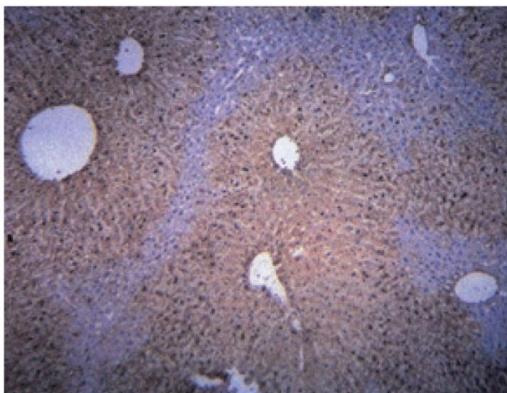
Approved drugs are frequently recalled from the market, and clinical trials of experimental drugs are often terminated early because of drug-induced liver injury. When 36 inbred strains of mice were given large doses of acetaminophen, large strain-dependent variations in drug-induced liver injury were found

(assessed by histology and by elevated serum levels of the liver enzyme alanine aminotransferase (ALT)⁸.

High doses of acetaminophen in humans (15 g) are toxic through centrilobular necrosis.

As can be seen in F8.15A, acetaminophen adducts are formed specifically in this region.

F8.15A.
Acetaminophen injury to the liver. Liver section from acetaminophen treated mouse, 300 mg/kg at 4 hours. Stained with brown antibody specific to acetaminophen adducts the centrilobular regions. Bluish regions are peri-portal. Josephy and Mannervik, 2006.

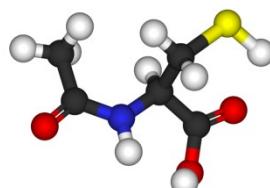


Quantitation of pathways (see F8.15):

Path 1: Sulfate 52%, Glucuronide 42%, Unchanged: 2% .

Path 2: 4% in toxic pathway when 70% of glutathione has been consumed.

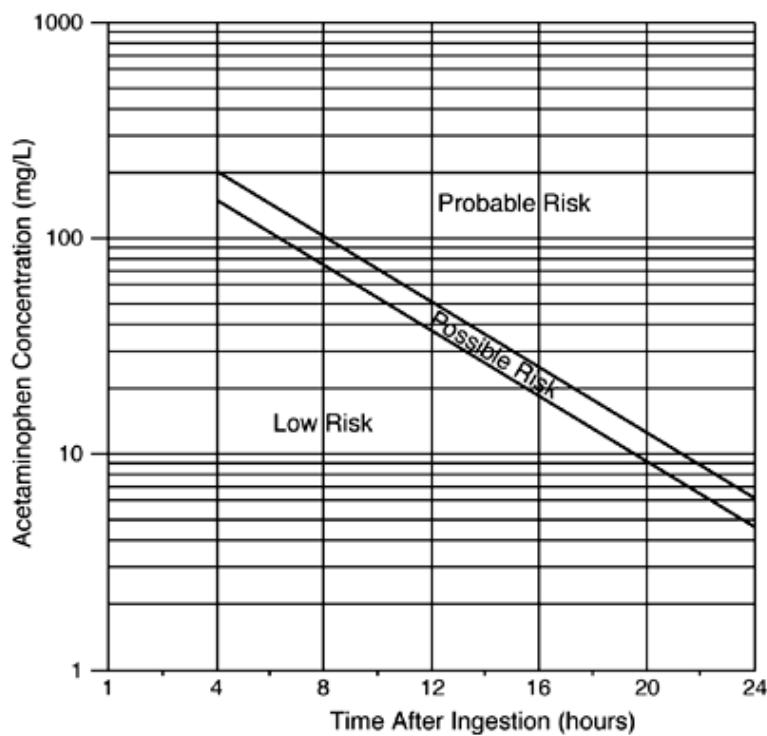
Oral N-acetylcysteine (NAC, shown), a precursor of glutathione, is the standard therapy for acetaminophen overdose. NAC is administered orally



with an initial (loading) dose of 140 mg/kg followed by 17 maintenance doses of 70 mg/kg every 4 hours^{3,7}. NAC has a foul smell and tastes like rotten eggs. Not surprisingly, nausea and vomiting commonly occur following administration. Peak plasma levels of NAC occur approximately one hour after an oral dose and at 12 hours post-dose it is undetectable in plasma. Despite a relatively low bioavailability of only four to ten percent, oral administration of NAC appears to be clinically effective. The biological activity of NAC is attributed to its sulphydryl group (yellow), while its acetyl substituted amino group affords it protection against oxidative and metabolic processes.

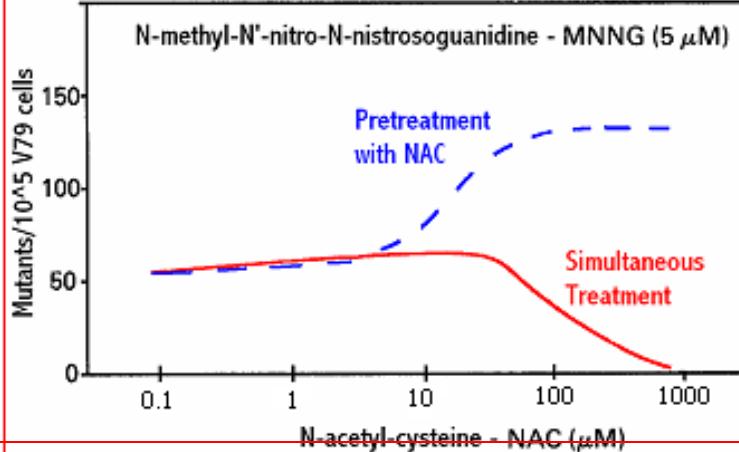
The nomogram below (F.8.16) is a nice application of toxicokinetics, but should not be used to *downgrade* the administration of NAC over time.

NAC is a good example of a compound which can have deleterious or protective effects, depending on its compartmentalization. In F8.17, the number of mutant cells produced as a function of NAC concentration is plotted for two cases: *pretreatment* and *simultaneous* treatment with NAC. In *pretreatment*, NAC is first metabolized to increased intracellular glutathione, which enhances activation of MNNG near the target molecule, after it enters the cells. This results in more mutants. In *simultaneous* treatment, MNNG is activated by NAC extracellularly, and the reactive intermediate harmlessly reacts with extracellular water.



F8.16. Rumack-Matthew nomogram for acetaminophen intoxication from serum values. *Rumack J. Toxicol Clin Toxicol 2002;40(1):3-20.*

Note that mice that do not express CYP2E1 do not suffer from



acetaminophen toxicity, while snakes are highly sensitive.

8.5.1.2. Halogenated Alkanes/Alkenes

These hepatotoxic chemicals (T8.18) also involve other target organs (T8.19). They are not toxic by themselves, but are

F8.17. Mutagenicity of MNNG depending on the timing and concentration of NAC treatment. *Romert and Jenssen, 1987.*

biotransformed into toxic intermediates: radicals and epoxides^{3,4}.

Carbon tetrachloride and chloroform result *in vivo* in specific cytotoxic intermediates (F8.20). The detoxification of the radicals (carbene, peroxy) and the intermediate (phosgene) involves their conjugation with glutathione (GSH) until stores of GSH are depleted. The isolation and identification of these unstable intermediates has been extremely difficult. Hepatic biotransformation proceeds too rapidly, but biotransformation by renal homogenates or fractions is much slower, and we now know all of the toxic products arising from carbon tetrachloride biotransformation.

T8.18. Some halogenated alkanes/alkenes.

Carbon tetrachloride	CCl_4
Chloroform	CHCl_3
Trichloroethane	$\text{CH}_2\text{Cl}-\text{CHCl}_2$
Trichloroethylene	$\text{CHCl}-\text{CCl}_2$
Halothane	$\text{CF}_3-\text{CHClBr}$
Freons	CCl_3F CCl_2F_2 $\text{CClF}_2-\text{CFCl}_2$ $\text{CClF}_2-\text{CClF}_2$

Despite the concerns about occupational exposure to such agents as carbon tetrachloride or chloroform (a possible human carcinogen), chloroform was used as a "safe" anesthetic (it did not explode like diethyl ether) until the hepatotoxicity was

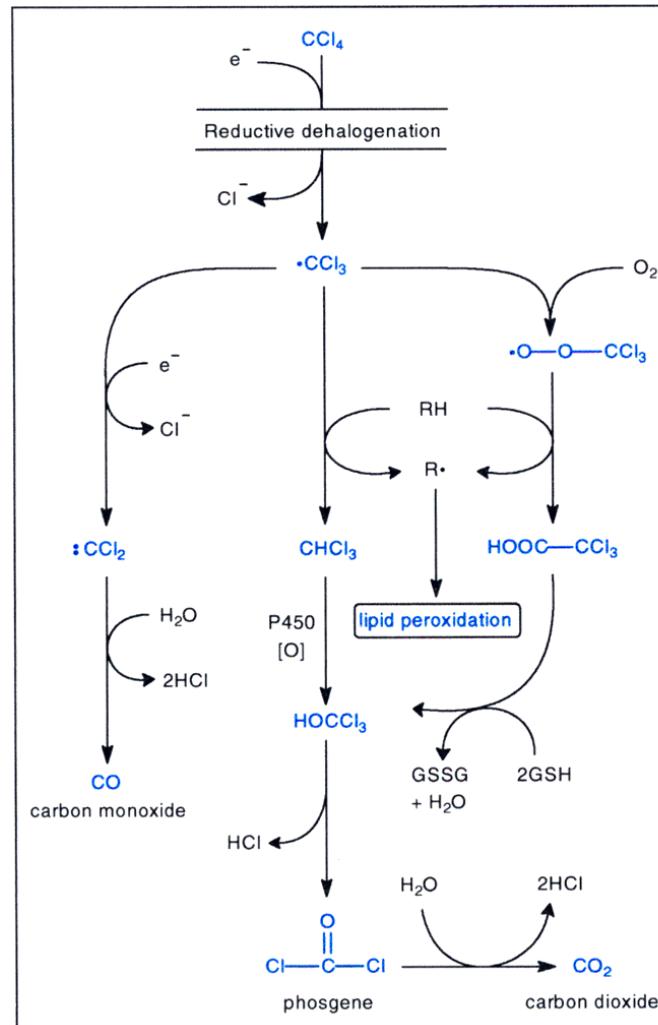
T8.19. Toxicity of haloforms. *on acute exposure when catecholamines are administered concomitantly. Other haloforms show the same property.

Compound	Hepatotoxic	Nephrotoxic	Cardiotoxic
CCl_4	X	X	
CHCl_3	X	X	
Halothane	X		
Methoxylfuran		X	
Freons			X*
Vinyl Chloride	X		
Perchloroethylene	X		

recognized. Carbon tetrachloride is still used as an antiparasitic agent (vermifuge) in third-world nations because it is cheap and effective, and can be administered orally in capsules.

Understandably, you should not give it too often (once a year), for fear of hepatic toxicity.

The signs and symptoms of halogenated alkane hepatotoxicity



F8.20. Reductive dehalogenation of CCl_4 to a trichloromethyl free radical that initiates lipid peroxidation.

include protracted nausea and vomiting, severe prostration, jaundice, enlarged palpable liver (right side below rib cage or below sternum) and elevated serum transferases.

Via a needle biopsy, one can see swollen cells and fatty degeneration, predominantly in the centrilobular area but radiating out toward the portal triads (the mid-zonal regions of the lobule), as coagulative necrosis.

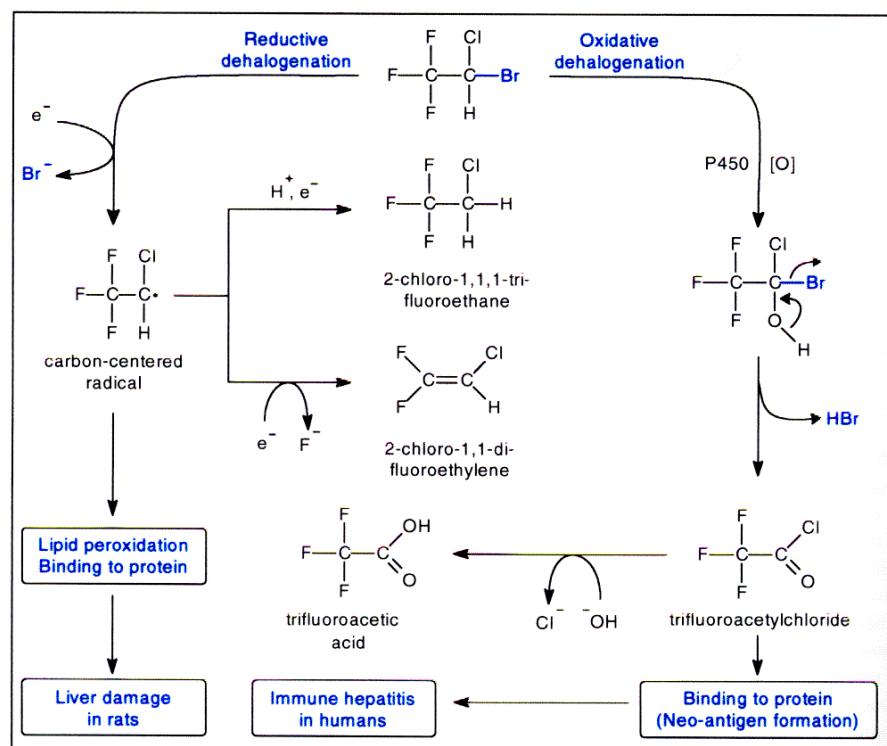
At necropsy, one sees an enlarged liver (hepatomegaly) with accentuated lobulation having a pale appearance or the classical "pearly" liver - surface cells outlined with a ring of lipid around each. Cells around the central vein have a pale appearance upon staining and contain large fat globules.

A gradient in necrotic damage is seen from the central vein out into the mid-zonal regions, most severe in the central vein region.

It is apparent that the Kupffer cells play some role in carbon tetrachloride induced hepatotoxicity⁵. Carbon tetrachloride appears to activate Kupffer cells by increasing their intracellular levels of calcium ions which, in turn, trigger the release of cytokines and eicosanoids (leukotrienes). Those are chemotactic factors for neutrophils which become activated, are attracted to the region, and secrete superoxide anion and other toxic mediators which amplify the inflammatory response leading to cell injury and death. It is important to appreciate that the above mechanism of hepatotoxicity is not unique to carbon tetrachloride but applies to all haloalkanes and alkenes and, perhaps, to other hepatotoxicants as well.

Back in the 1960s, there was considerable concern about the severe hepatotoxicity witnessed when halothane was first used as an anesthetic. Halothane was almost "finished" in the marketplace before it was realized that the anesthesia machines through which it was administered had copper kettles in which the anesthetic was placed before air was passed over it. The high cost of this anesthetic (250 \$ for 100 ml) meant that the residual anesthetic was kept, frequently left in the copper

chamber. Of course, halothane is covered with halogens (F, Cl, and Br) which react in the presence of copper to produce a toxic intermediate which the next patient receives when anesthetized. When the copper chamber was replaced by glass, hepatotoxicity dropped sharply. As yet, not all of the possible biotransformation products of halothane have been identified (F8.21).



F8.21. Activation of halothane by reductive and oxidative dehalogenation. Their role in rat and human liver toxicity.

Casarett & Doull.

8.5.2. Natural Hepatotoxins

Cyanobacterial blooms often take place in lakes during sustained warm spells in autumn. Cyanobacterial hepatotoxins can kill a drinker within 24 hours. The toxins attack a protein in the membrane of liver cells, and the cell membrane breaks down. Subjects die from internal bleeding.

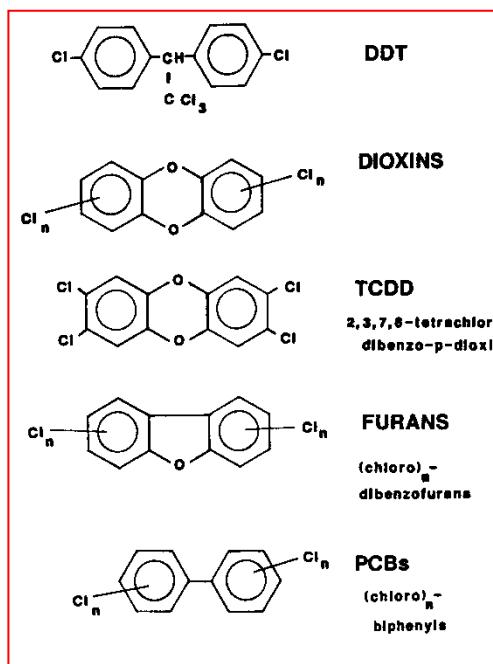
8.5.3. Persistence of Hepatotoxicity

Many chemicals can exert subtle effects on the liver, specifically those that the liver finds difficult to biotransform, or the liposoluble ones that penetrate cell membranes and are stored in the body for exceptionally long periods of time. Detectable blood levels of brominated and chlorinated dioxins can be demonstrated 35 years after exposure³. The effects of these agents during that period are uncertain. We all possess low adipose tissue stores of DDT and PCBs that are slowly released.

The oldest example of such agents is the insecticide DDT, not very toxic, difficult to biotransform, highly liposoluble, persistent for decades both in environmental food chains and in the human body. Much of the body burden is stored in the adipose tissue, with a small amount circulating in the bloodstream.

Polychlorinated biphenyls, dioxins, and furans (F8.22) are not terribly toxic to the liver but are very liposoluble and persistent *in vivo*.

TCDD is the most toxic of the congeners to animals. The liver treats such agents as if they were a high level exposure situation, even though the level may be quite modest. If the liver cannot biotransform the agent, enzymes are induced in the hope of eliminating them. This is the liver's only response to a situation that is *perplexing* to it. However, the resistant chemicals remain and the elevated enzymatic activity



enhances the biotransformation of other chemicals to which the subject may be exposed.

F8.22. Structures of persistent halogenated aromatic hydrocarbons capable of long-term induction of tissue enzymes.

The Phase I and II enzymes will not decline back to normal, since the agent persists in the bloodstream, maintaining an *alert state*.

8.6. Tests of Liver Function

Tests of hepatic injury are serum enzyme tests (below), hepatic excretion tests (sulfobromophthalein, indocyanine green), tests on alteration of the liver's chemical constituents and histological analysis of liver injury.

Current hepatic tests in the clinic are blood tests that detect the presence of damage or inflammation, rather than how well the liver functions.

Severe hepatic damage results in leakage of a number of cytosolic (soluble) enzymes (SGOT, SGPT, LDH, Sorbitol DeHydrogenase, etc.) through the hepatocellular membrane, with sharp elevations of these species in serum where only low

levels are usually found. This may persist for prolonged periods of time. Particularly with PCBs, one will find elevated serum triglycerides and sometimes cholesterol, as well as serum enzymes and dermatological acne for months and even years^{1,2}.

An example of carbon tetrachloride-induced hepatic damage in rats (F8.23) shows which enzymes are diagnostic for toxicity. The same can be seen in humans. However, hepatocellular damage occurs at different rates with different chemicals, and one blood sample may not yield the desired diagnostic results. For example, benzene will produce damage (and enzyme release) in approximately 8 hr whereas, with carbon tetrachloride, it takes more than 24 hr before significant cellular damage and enzyme leakage occurs.

Fasting Serum Bile Acid (SBA) determination can be used clinically in the diagnosis and prognosis of liver disease in conjunction with standard liver function tests. Because of the sensitivity of SBA determination as compared to standard liver function tests, SBA offers significant additional diagnostic information concerning liver function, especially in minor hepatic derangements. It is of particular benefit in the determination of hepatic dysfunction as a result of chemical environmental injury.

Liver injury as a result of occupational or environmental exposure to a wide variety of chemical substances can be determined to a much finer degree by SBA than by standard liver enzymes, especially when the liver has been only slightly damaged. In one study of individuals exposed to organic solvents, 73% of the exposed cases had elevated SBA levels, whereas increased levels of SGGT, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and bilirubin were observed respectively in 8, 3, 2, and 1% of exposed workers. These results and others support the hypothesis that standard

liver function tests are not sensitive enough to determine hepatic dysfunction caused by such organic solvents as toluene, xylene, acetone, styrene, n-butylacetate, n-butanol, ethylacetate, other aromatic hydrocarbons and ketones⁶.

There are a number of useful clinical tests beyond serum enzymes, such as:

- ⊕ Functional test: clearance of bilirubin or dyes (bromo-sulfo-phthalein, indocyanine green).
- ⊕ Albumin is a major protective blood protein formed in the normal liver. Levels below 3.5 mg/dL are abnormal.
- ⊕ Prothrombin Time: clot formation is indicative of liver function. Blood from the patient is oxalated to prevent coagulation. Later, large amounts of calcium and tissue extract are mixed in. Prothrombin starts transforming to thrombin, leading to coagulation. Normal is about 12 seconds. Deviations of more than 2 seconds are abnormal.
- ⊕ Liver scan using radio-opaque chemical.

8.7. In Vitro Liver Experimentation

A number of products are available on the market to experiment *in vitro* on liver function. Fresh animal hepatocytes, cryopreserved hepatocytes from various species (shown), individual and pooled microsomes or S9 fraction.



Cryopreserved hepatocytes should be stored at less than -150°C to assure long-term viability. Microsomes and S9 should be stored at less than -70°C to preserve P450 enzymes.

Primary hepatocytes, when cultured, begin to lose liver-specific gene expression within just a few days, unless co-cultured with non-parenchymal cells (non-liver) such as fibroblasts or endothelial cells⁹.

T8.23. Hepatic damage in male rats with 0.1 ml/kg of CCl₄. Serum enzyme activity, 3 days post treatment.

Enzyme name Target Tissue	Control	Treated	Increase
Alanine Aminotransferase (ALT, formerly SGPT) Liver & heart	42 ± 4	440	10x
Aspartate Aminotransferase (AST, formerly SGOT) Liver & muscle	91.2 ± 11	720	8x
Alkaline Phosphatase (ALP) Bones & liver	917.2 ± 51	2560	3x
Acid phosphatase	20.9 ± 2	43.8	2x
Creatine (phospho)kinase (CPK) Cardiac/skeletal muscle	218 ± 61	284	
Gamma Glutamyl Transaminase (SGGT) Cholestasis			After alcohol intake
Lactate Dehydrogenase (LDH) Muscle, Erythrocyte, liver	108 ± 31	1019	9x

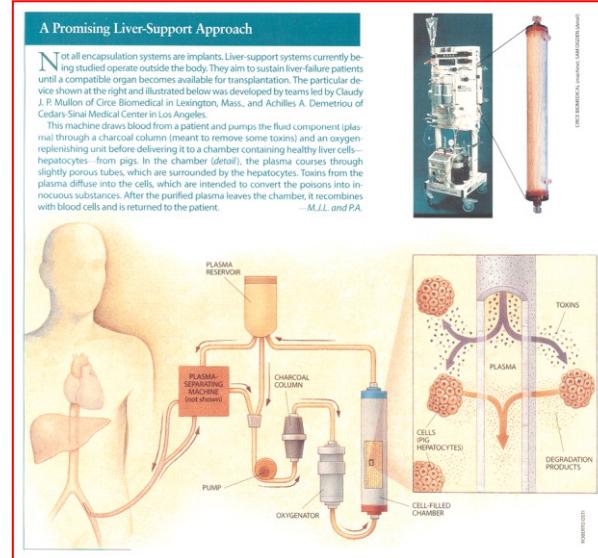


8.8. Temporary Liver Support

Liver regeneration may actually occur from bone marrow cells, as was recently found. First, bone marrow stem cells may serve as a source for the replacement of endothelial cells. Second, hematopoietic cells, including lymphocytes, neutrophils, macrophages, and platelets, may provide crucial factors required for efficient healing of damaged liver.

When regeneration fails, temporary liver support (F8.24) can be very important. In acetaminophen intoxication, for example, if the patient survives, complete liver recovery *can* occur.

ALP and SGGT reflect cholestatic injury more effectively than necrotic injury. LDH, AST and ALT are more sensitive to cytotoxic hepatic injury. These enzyme determinations are less sensitive than histological pathology.



F8.24. Research in Temporary Liver Support.

8.9. Case Study: Acute Dimethyl Nitrosamine Poisoning

(modified from Williams & Burson)

In Omaha, Nebraska, the county health department became concerned because there seemed to be an outbreak of a new disease; five people had suddenly become ill. The sick patients experienced vomiting, severe abdominal cramps, diarrhea, and two of them developed bruising. The first person had become ill on a Sunday morning while painting his house. Later in the day, his 2½ year-old daughter became sick. During the afternoon, his wife's sister, her husband, and another child visited them. They stayed for a little while, drank some lemonade while there, and left. At six o'clock that night, they had also started feeling ill. The man who had become ill early Sunday morning went to emergency rooms and then to a number of physicians, but he was repeatedly told he had the flu and should go home, rest, and take aspirin. Later, he developed severe nosebleeds; his nose was packed twice in emergency rooms early in the week. After each episode he was sent home again. He persisted in seeing physicians and, finally, on Thursday of that week, he walked into a physician's office and collapsed. At that point he was admitted to a hospital. On admission he showed abnormal liver function tests. The fact that his bilirubin was elevated also indicated a liver problem. The reason for the bleeding problems was that his platelets were severely decreased; there were about 6,000/mm³ instead of the 150,000-300,000/mm³ or more that would be normal.

Because he became comatose, the child of the family who had visited the home on Sunday afternoon had already been admitted to another hospital. It took the hospitals a while to recognize that there might be a connection between the two cases. Once that was established, the county health department was called. The county health department then started looking at the other people who had visited the home and eventually established that of this group-a total of ten people who had been involved-five were ill and all of them had abnormal liver function tests. They all had low platelet counts. Not all of the people had platelet counts as low as those of the man or of the child who had visited the home.

After interviewing all who had visited the home on the Sunday when the illnesses were first noticed, the health department investigators concluded that the vehicle for the illness-causing agent must have been food or drink. The only food item all the ill people had consumed was lemonade. The parents of the man who had first become ill had visited the home, but while they were there, they did not consume any food or drink; they did not become ill. A sister living with the first family did not drink lemonade and did not become ill; and a baby did not eat any foods because it was being breastfed. The fact that the baby did not become ill eliminated to some extent the possibility of a highly contagious illness (except possibly a food-borne organism).

The lemonade therefore seemed implicated. By this time, of course, the family had discarded any remaining lemonade (always a problem in these types of cases).

The child died. The next day the man died. After that, the CDC was called. The county health department wanted to know what they should do, what kind of tests they should run, and what kind of samples they should take from the man. The CDC suggested that, in addition to taking tissues as is normally done for microscopic examination, they should also freeze tissue so that chemical analysis could be performed on the tissue if it became necessary to do so.

The autopsies of the father and child produced two common findings. One was that severe liver damage was the cause of death. In addition, there was extensive bleeding owing to the decrease in platelets. There were no other significant findings. No other organs were specifically affected. Review of the liver sections and other organs did not suggest an infection, because there was no inflammatory reaction in the tissues.

The question was, what type of chemical would produce these toxic effects? Quite a number of chemicals damage the liver, but they usually affect other organs as well. Because the investigation implicated the lemonade and the people had not complained about the lemonade's tasting peculiar, the chemical had to be something that did not taste strange and something that was water soluble. These observations somewhat limited the number of compounds. The substance also had to be relatively toxic so that a person would ingest a lethal dose by drinking a single or a half glass of lemonade in which this material was dissolved.

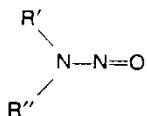
At the CDC we examined a number of the more common compounds that cause liver necrosis. We did not include in the list of compounds substances that, like carbon tetrachloride, severely affect the liver but also affect the sensorium. Carbon tetrachloride also has some effect on the kidneys but would be detected by people drinking lemonade. Portions of the liver tissues were examined for arsenic and yellow phosphorus.

None of this made very much sense. We searched further. Some people are sensitive to acetaminophen, a pain medicine. It can damage the liver, but typically not everyone in a family suddenly becomes ill. Usually there is a history of the patient's having taken this medication, but these patients had not taken acetaminophen.

Aflatoxin, a mycotoxin and another hepatotoxin was also a suspect, but it would be very difficult to have access to this type of compound. Furthermore, the microscopic appearance of the liver did not suggest aflatoxin. However, certain types of alkylating agents that are either used in cancer chemotherapy or cancer research might selectively damage the liver and could cause the severe centrilobular liver necrosis.

In talking to the county health department, I asked for additional information about the family to determine whether they would have had any contact with people in a cancer research institute or a hospital. The county health department thought this was a peculiar idea, but passed the request on to the police. A police officer in Omaha went to his files and found that this family did indeed know a man who was now working at the Eppley Institute in Omaha, which is a cancer research institute.

Five years earlier this man had had a love affair with the wife of the man who later died, and had confronted the family with a gun; shots had been fired. He had then been sentenced to prison and was free on parole. Because he held a degree in biology, he was able to obtain a job at the Eppley Institute. His job was to mix diets for cancer research studies in animals, and one of the compounds he was working with was the alkylating agent dimethyl nitrosamine. The essential chemical structure of dimethyl nitrosamine is



Generalized structure of N-nitroso compounds

Dimethyl nitrosamine was eventually established as the compound that caused the illness and death in the family. While the family was away from their house, the suspect had climbed into a back window and added the

poison to a pitcher of lemonade found in the refrigerator. His intention was to cause the people to have cancer; he wanted to watch them die. However, he picked a chemical that was also very acutely toxic. The oral LD₅₀ in rats for dimethyl nitrosamine ranges between 27 and 41 mg/kg of body weight. The calculated lethal amount required for a child is less than one gram and for an adult roughly three grams.

Dimethyl nitrosamine is a yellow oil, so it mixes very well with lemonade. It is water soluble. It is somewhat more stable in a slightly acid environment. Other nitrosamines were examined to determine whether some other compound could have produced the effects evident in this case. They were ruled out for a number of reasons, and they were also not available at the institute. Many are not nearly as toxic, and the other more toxic nitrosamines do not specifically cause the selective centrilobular liver necrosis.

Dimethyl nitrosamine is very rapidly metabolized and excreted; therefore, five days after poisoning (the time that elapsed before investigation of this case began), all of the material would be metabolized and excreted and would not be found in the body. Nitrosamines and other alkylating agents do one thing by which they can be traced, however. They methylate nucleic acids, such as, for example, guanine.

There is now a test that has been developed, using high pressure liquid chromatography, by which methylation of guanine can actually be measured. Early in our investigation liver tissue from one of our patients was frozen. A total of eight tissue samples were then blindly submitted for analysis: seven controls and our sample. The chemist analyzed the eight samples and identified methylated guanine in the liver specimen that turned out to be our sample. Products like methylated guanine are also excreted in urine and would also probably be measurable in urine specimens. It is presently not possible to demonstrate methylation with acute yet nontoxic doses because the method is presently not sensitive enough. The amount of the concentration found in the liver of the adult male was as high as those demonstrated in rats that have been acutely poisoned with dimethyl nitrosamine,

however. We were able to show this in court and the man who poisoned the family was convicted.

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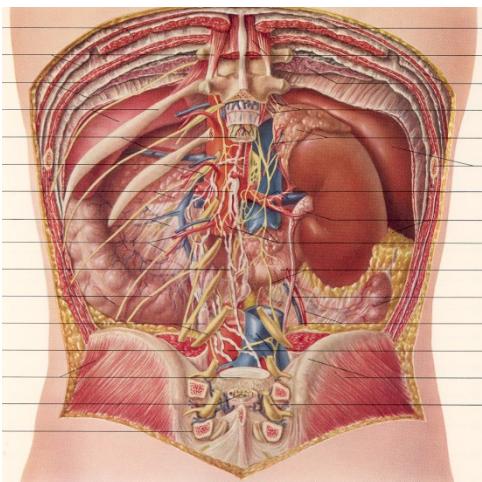
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9. Nephrotoxicity

9.1. Roles of the Kidney



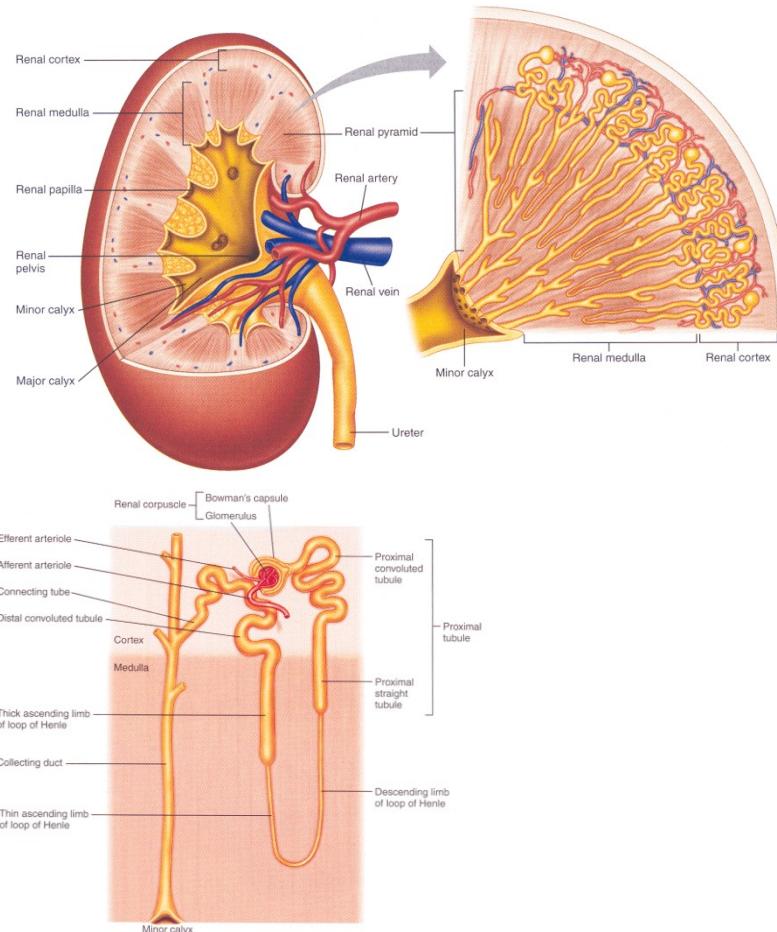
The kidney is the main organ responsible for the expulsion of endogenous metabolites and xenobiotics.

F9.1. Gross anatomy of the kidney. *Atlas of Normal Anatomy*.

Its functions are:

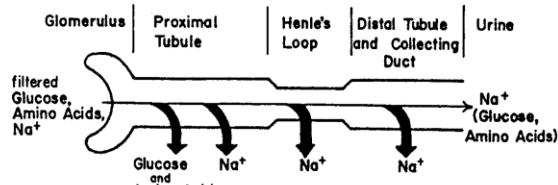
- Glomerular filtration, (a passive process),
- Tubular reabsorption,
- Tubular secretion (an active process),
- Regulation of extracellular volume,
- Regulation of electrolyte balance (a familiar example of imbalance results in muscle cramps),
- Acid-base regulation,
- Calcium metabolism.

There are approximately 10^6 nephrons per kidney, with a total length of approximately 120 km. There are indications that mothers restricted in protein give rise to offspring with fewer nephrons. The remaining overworked nephrons release hormones and retain sodium. The offspring is then vulnerable to hypertension³.

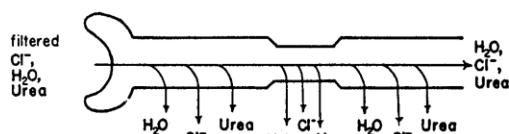


F9.2. Kidney anatomy. *Germann & Stanfield, 2002*.

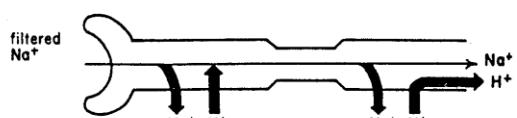
The length, the large surface area and the thin barrier between the lumen of the tubule and the extensive capillary blood supply (F9.2) insures a rapid transport of many substances. Approximately 20 to 25% of the blood pumped out of the heart each minute is routed through the kidneys. They filter the entire blood volume every 4 to 5 minutes.



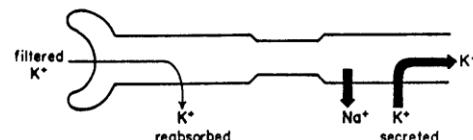
Glucose and amino acids are excreted only when the amount filtered exceeds saturation of active transport. Na is excreted in direct proportion to intake. The heavy arrows indicate active transport, the light arrows diffusion.



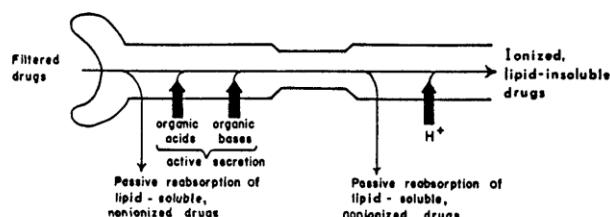
Diffusion of water, chlorine ions and urea along osmotic gradients established by active transport, particularly of sodium. With ADH, water is excreted in direct proportion to intake. Without ADH, water is not reabsorbed in the distal tubule and collecting duct. 55% of the urea is reabsorbed. Chlorine and sodium are excreted equally to maintain electroneutrality.



Acidification by active reabsorption of sodium in exchange for protons secreted into urine.



Potassium is almost completely reabsorbed in the proximal tubule. It is excreted in the distal tubule, in exchange for sodium.



Lipid-soluble and non-ionized drugs are passively reabsorbed. In distal segments, the secretion of protons favors reabsorption of weak acids (less ionized) and excretion of weak bases (more ionized), compatible with the Henderson-Hasselbalch equation. Active secretion of organic acids and bases occurs only in the proximal segment.

< F9.3. Functions of the Kidney Tubule.

The kidney loses 20% of the plasma volume through the glomerulus, which acts like a sieve, and recuperates the needed components later, glucose at a rate of 99.96%, using the *proximal* and *distal* tubule. Therefore, the overall personality of the kidney is that it is a *recycling organ*.

In F9.2, note that the medulla consists only of tubular structures for blood and urine and that the glomeruli are located in the cortex.

The nephron is a very active tissue, as glomerular filtration occurs on-going with tubular secretion and reabsorption. The kidney has the same enzymes (Phase I, II) as the liver, but with only 20% of the activity. While low exposure levels of toxicants probably pose no problems for the kidney, moderate and high levels might.

9.2. Physiology

Xenobiotics biotransformed into water soluble products will most likely be excreted by passive filtration through the glomerulus and into the urine. By contrast, lipid-soluble, nonionized chemicals will be passively re-absorbed into the blood from the lumen.

The various segments (A-E) of F9.3 describe components of tubule functions.

A – All glucose and amino acids are recuperated, unless transport mechanisms are overwhelmed. Normally, only 0.04% of glucose is lost.

Sodium is actively reabsorbed in the tubule (A and C) and hydrogen ions are actively secreted as a substitute (C), so the pH of the urine becomes acidic (from 7.4 to 6.5-6.0).

Cl and water follow Na (B), itself excreted in proportion to intake.

pH values in the urine range from 4.7 to 8, with a normal of 6. The control results from the combined effects of the various buffer systems, particularly the carbonate, phosphate and ammonia buffer systems.

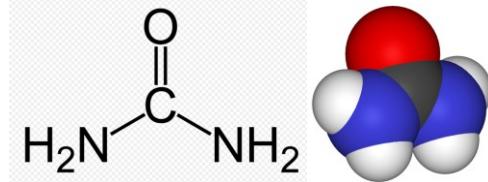
For organic acid excretion, a basic urine is desirable; for organic base excretion, acidic urine is desirable.

For example, the glucuronide of the xenobiotic phenol is more polar and more water soluble at pH 6.5 than at pH 7.4 (the pH of the blood), and so can be more easily eliminated.

For organic bases, the acid pH of the urine enhances the polarity of the excreted product and insures that it remains in the fluid, and is not re-absorbed.

F9.4. Structures of the Kidney. *Biological Structures.* >

B - Urea, formed in the liver from metabolism of proteins, has



a pKa of 0.1. According to the Henderson-Hasselbalch equation, a minuscule amount is non-ionized in the

plasma, but lowering the urine pH allows for somewhat increased passive reabsorption. Ultimately, about 55% of urea is reabsorbed. Its usefulness may be that it acts as an anti-oxidant in the blood.

E – Many toxicants are secreted in the proximal tubule, but can be reabsorbed, if not ionized, all along the tubule.

Some detoxification products, particularly those conjugated to glutathione, may be partially reabsorbed and may be toxic at the point of entry. Thioethers (R-C-S-C-R) are particularly nephrotoxic.

The kidney

Two sections of a Bowman's capsule with glomerulus. $\times 650$

Here the blood is filtered under its own pressure (ultra-filtration). The glomerular filtrate (plasma minus blood cells and proteins) passes into the capsule lumen. During the transport of the filtrate through the uriniferous tubules and the loop of Henle, selective reabsorption of useful materials (e.g. water, glucose and salts) takes place. The remaining filtrate (urine) is led to the collecting duct and on to the ureter.

start of uriniferous tubule
capsule lumen
glomerulus

capillary

transverse section of uriniferous tubule
transverse section of collecting duct

glomerulus in Bowman's capsule

loop of Henle
capillary

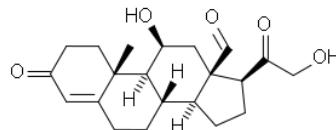
Transverse section of the ureter. $\times 125$

fat tissue
muscle layer
epithelium
ureter lumen

connective tissue with blood vessels

The tubular processes of secretion are controlled by a number of endogenous substances.

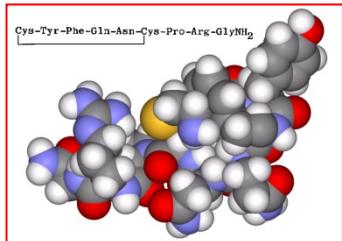
9.2.1. Aldosterone



It controls blood volume and therefore maintains blood pressure, by enhancing salt reabsorption in the tubule, which

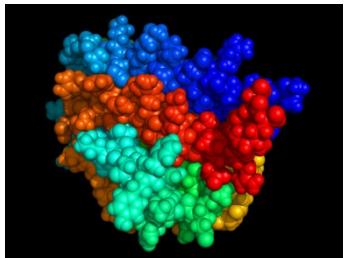
is followed by a corresponding water intake. Renin, from the juxtaglomerular cells, changes angiotensinogen to angiotensin I, to angiotensin II and finally to aldosterone, which is issued from the adrenal cortex.

9.2.2. Anti Diuretic Hormone (ADH)



Also known as vasopressin, controls urine concentration by increasing *reabsorption of water* in the distal tubule.

9.2.3. Renal Erythropoietin Factor



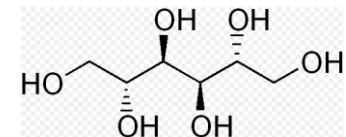
This factor increases RBC production and explains why chronic kidney failure often leads to anemia. Erythropoietin is produced in the proximal tubule cells, the same area where lead is known to accumulate in the kidney⁸.

9.2.4. Metabolism of Calcium

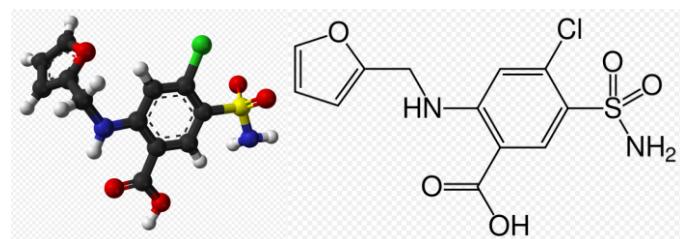
Calcium concentration in the blood is controlled by parathyroid hormone, which prevents it from falling too low, and by calcitonin, which prevents it from going too high. But there is only 1 g of Ca in extracellular fluid. In diarrhea, several grams of Ca can be passed in the feces each day. Therefore, bone acts as a Ca buffer. Parathyroid hormone secretion allows complete reabsorption of Ca in the kidneys.

Diuretics are medical agents used to treat edema (compartment syndrome, brain swelling) and hypertension (which can lead to kidney disease). There are a number of categories of diuretics:

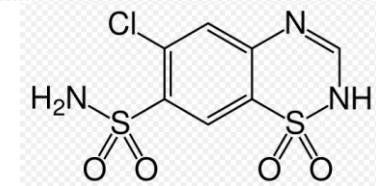
osmotic agents (mannitol),



loop agents
(furosemide),



thiazides (chlorothiazide shown),



aldosterone antagonists or ADH antagonists, that all act at various points along the tubules.

9.3. Pathology

Change in kidney function is often described using *creatinine clearance* as an index, and the Cruikshank formula:

$$\text{Creatinine Clearance} = \frac{(140 - \text{Age}) \times \text{Body Weight}}{72 \times \text{Serum Creatinine}}$$

Creatinine clearance in ml/minute, age in years, body weight in kg, serum creatinine in mg/dl. For women, multiply the figure by 0.85.

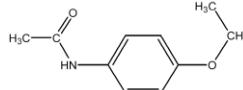
From a peak of kidney function at about 20 years of age, the creatinine clearance decays (because fewer nephrons are functional) such that by 60 years of age about a quarter of kidneys are considered defective, and the organs would cease functioning entirely at age 140.

The glomerulus is most vulnerable to immune complexes. The proximal tubule is vulnerable to halogenated hydrocarbons, heavy metals, antibiotics, antineoplastics and mycotoxins. The Loop of Henle is specifically vulnerable to fluoride ions.

Clinically, two entities are often distinguished.

✚ **Nephrotic syndrome:** characterized by proteinuria. Inducible by exposure to lead or heroin, as well as prolonged standing and strenuous exercise. Typical presenting features of nephrotic syndrome are marked edema, proteinuria, hypoalbuminemia, and usually hyperlipidemia.

✚ **Nephritic syndrome:** characterized by hematuria. It can be induced by mercury and phenacetin (shown).



9.3.1. Glomerulus Pathology

Pores of the normal glomerulus are about 0.01 μm, 100 times larger than those of capillaries in skeletal muscle. They limit molecule penetration to about 70,000 Daltons.

The filtration of albumin, a negatively charged molecule, is more restricted than neutral dextran of equivalent size. Loss of the fixed negative charge of the glomerular capillary wall results in enhanced filtration of anions. With loss of charge discrimination in *glomerulonephritis*, the capillary wall becomes size-selective only.

Proteinuria is often the first indication that disease is present. Normally there is a net protein excretion of 40 to 80 mg/day. Abnormal proteinuria is a protein excretion in excess of 150 mg/day. The classic intoxicator specific to the glomerulus is the immune complex. Toxic action in the glomerulus leads to decreased filtration rates, for example, with some drugs (gentamycin, kanamycin) or increased albuminemia (puromycin).

9.3.2. Tubule Pathology

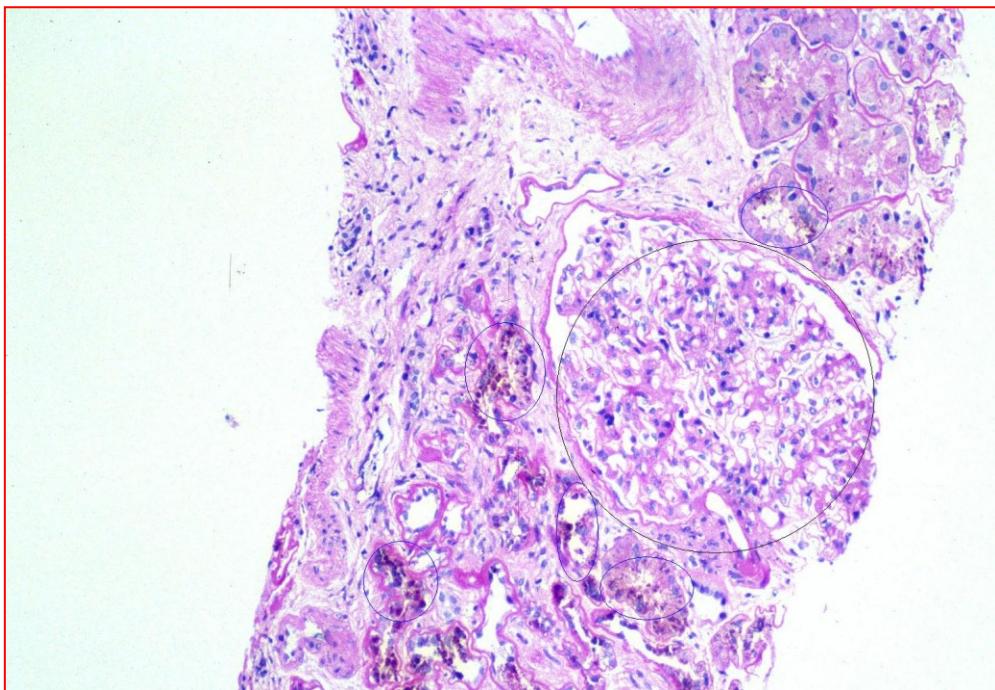
The tubule cells are subjected to high toxicant concentrations, even to possible crystallization.

In *shock*, reduced blood flow to the kidney does not allow the high tubule metabolism to be sustained, and tubular cells go into apoptosis. Normally, neighboring cells will stretch over the missing ones within a couple of hours, but with more apoptosis, serious lesions occur.

Toxicants (heavy metals, antibiotics, barbiturates, organic solvents) also can produce this effect, called *acute tubular necrosis*. Reduced urine flow causes buildup of toxic wastes in the body, leading to death.

Reabsorption of glucose and amino acids can be impaired by Cd, Pb, Hg, leading to glycosuria and aminoaciduria. Secretion of H^+ , K^+ and xenobiotics can also be impaired.

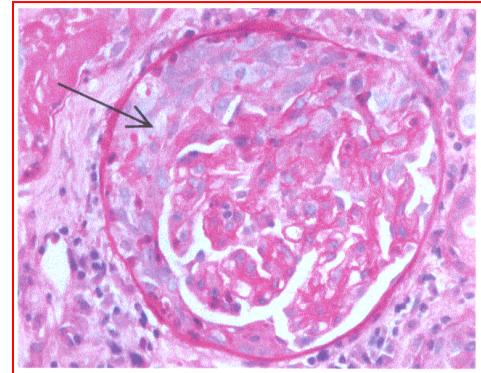
One major site of nephrotoxicity is the proximal convoluted tubule (F9.2), regardless of the nature of the toxicant. There are three reasons for this...



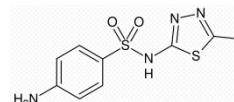
F9.5 In patients with sickle cell disease, who present with chronic renal disease and proteinuria, marked glomerular enlargement is present, often with mesangial expansion and segmental sclerosis. This glomerulus (thin black circle) shows glomerulomegaly and mesangial expansion. There is abundant brown pigment, representing broken down hemoglobin, in surrounding tubules (thin blue ovals), and interstitial fibrosis and vascular sclerosis.

Hematoxylin and eosin, x100. Atlas of Renal Pathology, National Kidney Foundation.

F9.6. Epithelial crescents (arrow) are found in a majority of the glomeruli in kidneys affected by rapidly progressing glomerulonephritis. *Scientific American Medicine.*



- (1) the content of the tubules becomes concentrated, as water is recycled (97.5% of the water is recovered). Cells lining the tubules are irritated by toxicant concentration, and the formation of crystalline structures from marginally soluble toxicants,
- (2) detoxification and unmetabolized products are drawn into the cells of the proximal tubule along with water, and remain there while water moves on into the bloodstream,
- (3) de-conjugation biotransformation, e.g. de-glucuronidation, de-sulphation yield uncovered reactive intermediates which cause cell damage.



A good example is the drug sulfamethizole, introduced many years ago. Its advantage over sulfanilamide is that it is not extensively metabolized *in vivo*, and exerts a prolonged action. However, one has to "push fluids", because sulphamethizole can precipitate in the tubule, causing laceration to the border cells, and bloody urine. This problem can be avoided by drinking a lot of water to keep the solution diluted, so that precipitation does not occur. There are also active secretory mechanisms located in the proximal tubule, one for organic bases and one for organic

acids (F9.3E). As with any active process, carrier protein and enzymes are involved, energy is required, and these secretory pathways can be saturated. Any damage to the proximal tubular cells by agents in the lumen can affect the active secretion process.

Effects of chemical exposure on renal function can be very insidious and subtle, evading detection for a long time. This is partly due to the redundant capacity in the kidney, where only about 25% of nephrons are used at any one time. An increased fluid load will bring additional nephrons into play, but after the "crisis", the extra nephrons reduce their activity. Considerable damage can occur in the kidney, with complete loss of many nephrons, before no reserve capacity remains. By that time, the damage may not be reversible.

9.4. Toxicity

9.4.1. Heavy Metals

Nephrotoxic Metals: Cadmium, Lead, Mercury, Arsenic, Bismuth, Chromium, Platinum, Thallium, Uranium.

The kidney is the main route of heavy metal excretion from the body, and most heavy metals are nephrotoxic.

At relatively low levels, a variety of signs and symptoms appear: glycosuria, aminoaciduria and polyuria, pointing to problems in efficient reabsorption in the proximal tubule.

At higher levels and at later stages in chronic exposure, elevated Blood Urea Nitrogen, renal necrosis, and anuria will precede death.

The histological picture is one of necrotic proximal tubules with the adjacent lumen filled with proteinaceous material.

Before toxicity is observed, the kidney will retain significant amounts of heavy metals, some (Hg, Pb) bound to various intra-cellular proteins, e.g. lysosomal acidic lipoproteins followed by a lysosomal endocytosis of these metal-protein complexes. The mitochondria are particularly susceptible to damage, and one sees autophagy of damaged subcellular organelles and extrusion of denatured proteins, all possibly protective reactions.

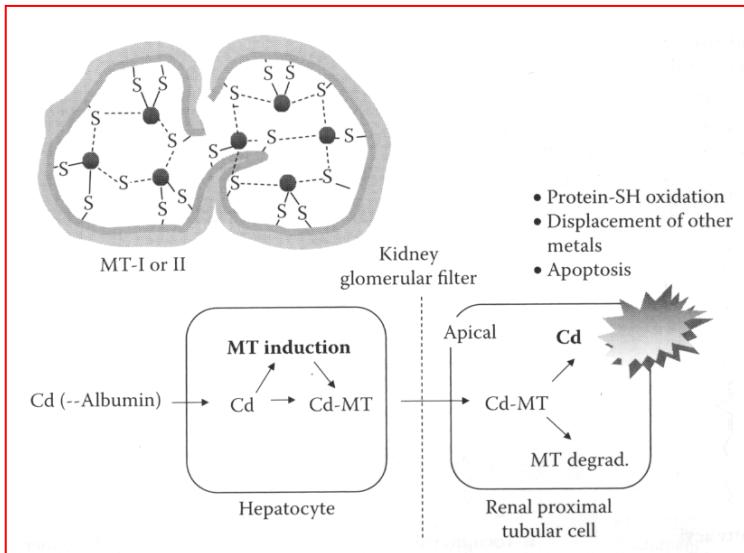
9.4.1.1. Cd

The kidney is the most sensitive organ to cadmium. About 5% of the US population has enough exposure to cadmium to injure the kidneys. The liver is the main synthesizer of a small (6,500 Dalton, 61 amino acids, 20 of which are cysteine) specific protein, metallothionein (MT), rich in free -SH groups, which binds (chelates) up to seven atoms of Cd, as well as Zn and Cu. The Cd-MT complex is stored in soft tissues of the body such as liver and kidney (which contains 10 times more than the liver). Metallothioneins are a surprisingly diverse group of molecules, but they are spread in a vast range to taxonomic groups, from prokaryotes and up. Metallothioneins have diverse metal-binding preferences. The rat's MT1 prefers binding divalent metal ions (Zn(II), Cd(II),...), while yeast CUP1 prefers binding monovalent metal ions (Cu(I), Ag(I),...).

When exposure to Cd exceeds the ability of tissues to synthesize MT and to store the Cd-MT complex, toxicity occurs above 300 µg Cd/g of cortical tissue. The complex is excreted via glomerular filtration, but can be reabsorbed in the proximal tubule. It was once thought that free Cd in tubular cells was the toxicant, but more recent evidence shows that the Cd-MT complex is toxic.

Cd normally accumulates in kidneys until age 50. Its half-life may be as long as 30 years. It is recommended that Cd intake not exceed 70 µg/day.

Progressively, low molecular weight proteinuria appears and cells concentrating Cd are lost, the Cd then evacuated in the urine. Chronic intoxication produces irreversible renal failure.



F9.7. Cadmium toxicity. Boelsterli, 2007.

Cd also produces brittleness as it replaces Ca in bones.

Cadmium has worrisome estrogenic effects in cell culture. Data from rats show that cadmium can act as an estrogen mimic in the whole animal, inducing conditions ranging from uterine hyperplasia to early onset of puberty⁵.

Cd is important in electroplating and color pigments. The greatest concentrations are found in the liver and kidneys of animals. Shellfish often have high concentrations.

There has recently been concern in Sweden over body burdens

of Cd from consumption of vegetables. Gastrointestinal absorption is 6%. Smoking doubles the daily Cd dose. Prolonged exposure to moderate levels of Cd in rice (0.3-1.0 µg/g) resulted during and after WW II in a strange muscular condition known in Japan as Itai Itai Byo (or ouch-ouch disease). It is characterized by deep muscle and bone pain of considerable severity, and bone deformation. Renal effects include proteinuria (particularly low molecular weight proteins), aminoaciduria (with elevated proline and hydroxyproline, suggestive of problems in bone metabolism), glucosuria and reduced absorption of phosphate and enhanced excretion of calcium (hypercalcuria)¹. The proximal tubule is the target site.

The effects were seen predominantly in older women, multiparous, calcium, and protein deficient. Cadmium absorption in the gastrointestinal tract can be enhanced by dietary deficiency of calcium, iron, and protein. Being older, and having had a number of children, these women would have been calcium deficient. Dietary problems during the war would have insured an enhanced absorption of Cd. Estimates exceeded 150 µg/day from food (mainly rice). The source of this Cd was paddy irrigation water taken from a river downstream from a metals mine, which allowed tailings and washings to run uncontrolled into the river for many years.

9.4.1.2. Pb

Lead causes two types of kidney toxicity in humans:

(1) proximal tubular damage reflected in glucosuria, aminoaciduria, hyperphosphaturia and problems of reabsorption,

(2) glomerular atrophy, interstitial fibrosis and sclerosis of vessels seen at prolonged, high level exposure, resulting in chronic nephritis.

Only the proximal tubular effects can be reversed with chelation therapy.

9.4.1.2.1. EDTA Therapy

Short term lead intoxication is usually reversible with EDTA (ethylene diamine tetra-acetic acid) therapy. In the early 1950s several deaths occurred from kidney toxicity after EDTA treatment. At that time, the dosage used was around 10 grams of EDTA per infusion, while it is now 3 grams.

EDTA kidney toxicity is related to the dose and the rate of infusion, which must be adjusted so that the infusion will not harm the kidney. If the patient is very elderly, has low parathyroid activity or is suffering from heavy metal toxicity, treatment should be modified to use less EDTA less frequently (once per week). The heavy metals that damage the kidneys during excessive infusion therapy are lead, aluminum, cadmium, mercury, nickel, copper and arsenic.

Tests of renal function should be performed before chelation therapy is started. In cases of renal impairment, lower EDTA dosage is indicated, with sufficient periods of rest between the infusions.

Long term exposure to lead may cause irreversible kidney dysfunction. "Saturnism" is lead induced gout because of increased uric acid levels.

9.4.1.3. Hg

Mercury is a classical nephrotoxin, used in experimental models of kidney failure.

The damage is primarily in the *pars recta* of the proximal tubule, with proliferation of the smooth endoplasmic reticulum (SER) and extrusion of SER "packets". Organic acid secretion (p-aminohippuric acid, PAH), predominantly in the *pars recta*, is reduced by Hg exposure. The basic toxic action of Hg is inhibition of the free sulphhydryl groups (-SH) of enzymes.

Acute poisoning: brief polyuria may be followed by anuria. If subject survives, polyuria follows, lasting many months.

Chronic poisoning: proteinuria, glomerular and tubular damage.

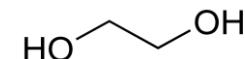
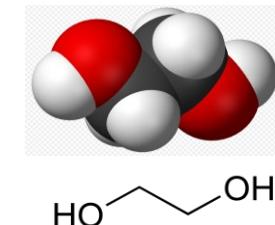
Morphologically, one sees a selective necrosis in the *pars recta* region, loss of brush border, (villi) from cells, with the formation of clumps of membranes in the cellular cytoplasm, vacuoles, distinct mitochondrial changes characteristic of impending necrosis with eventual necrotic destruction.

9.4.1.4. Cr

Chromium produces proximal tubular damage with glucosuria, but lower down than does Hg. Ischemic patches on the kidney surface are suggestive of a vascular effect.

9.4.2. Ethylene Glycol

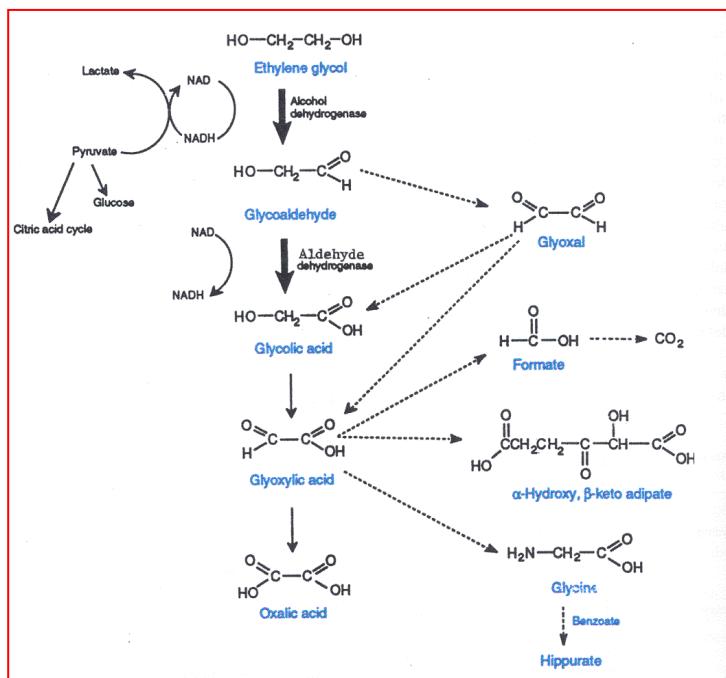
Ethylene glycol (car anti-freeze) is biotransformed both in the liver and in the kidneys to oxalic acid, but the kidney is particularly susceptible. Oxalic acid crystallizes in the lumen and in the cells of the tubule into calcium oxalate salt, leading to laceration of the tubules. Leaves of rhubarb contain large amounts of oxalate. Ethylene glycol proceeds through a number of oxidative reactions leading to acidosis (F9.8). The final product, glyoxylic acid, can be converted either into formic acid or oxalic acid. Oxalic acid may react with calcium ions to produce insoluble crystals of calcium oxalate which precipitate in the renal tubules, eventually producing kidney "stones" and tubular epithelial necrosis with lipid inclusions. Also visible, microscopically, is a thickening of the basement membrane in the glomerulus along with granular deposits.



100 deaths occurred in 1937 when ethylene glycol was used as a solvent for an oral sulphanilamide pharmaceutical preparation. Within two days of consumption, nausea, vomiting, intestinal cramps, diarrhea, and back pain in the region of the kidneys were experienced. These initial symptoms led to progressive hepatic

necrosis, renal tubular degeneration and death. Substitution of ethylene glycol by propylene glycol avoids all renal toxicity.

Lavage and **activated charcoal** can be used in the upper digestive tract to minimize absorption. The principal goal in such poisoning is to correct the acidosis (administration of bicarbonate), eliminate the unmetabolized ethylene glycol (by hemodialysis), and to block further tissue biotransformation by administering **ethanol** (20%) which will compete successfully for the enzyme alcohol dehydrogenase to block the formation of glycoaldehyde. The ethanol should be administered within 8 hr of exposure in acute cases (ingestion) and be continued for at least 5 days.



F9.8. Ethylene glycol toxicity. Casarett & Doull.

In addition to the renal effects, ethylene glycol has other target organs: CNS (depression, initially), muscle paralysis (CNS related), decreased tendon reflexes, convulsions and tetany (due to hypocalcemia), pulmonary edema and congestive heart failure.

Ability to concentrate urine is often the last property to be regained by the kidney after recovery from intoxication.

9.4.3. Organic Solvents

Carbon tetrachloride and chloroform act predominantly in the proximal tubule. Carbon tetrachloride causes severe hepatic

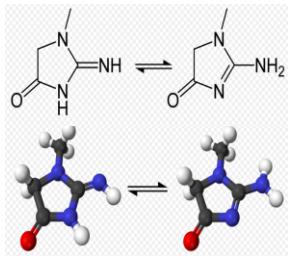
necrosis, but the cause of death is kidney failure.

Renal tubular disease can be caused by a variety of volatile organic solvents, most likely as a consequence of reabsorption into the proximal tubule and biotransformation there into reactive and toxic intermediates. Glomerular damage can also be produced by such agents, a slow progressive disease with an insidious onset seen in adults^{2,3}.

9.5. Tests of Kidney Function

The general medical indicators of kidney function are *Blood Urea Nitrogen* (catabolism of proteins, normal 2.9-7.1 mmol/l) and *serum creatinine levels* (from muscle metabolism, 60-130 mol/l). Elevated levels in the serum are indicative of kidney dysfunction, specifically low Glomerular Filtration Rate. The basic test of kidney function is *clearance*. Clearance can be higher or lower than the glomerular filtration rate if a substance is actively secreted or reabsorbed, and is routinely assessed by physicians to predict the rate of drug elimination in patients. BUN and serum creatinine are related to GFR (F9.9).

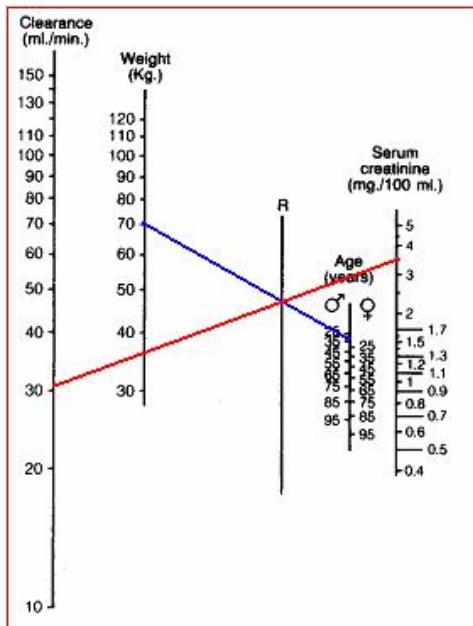
9.5.1. Creatinine Clearance



The rate at which a drug is eliminated by the kidneys is commonly proportional to the clearance of creatinine. It is not always convenient before starting treatment to wait for a creatinine (shown) clearance measurement, but it can be estimated by using the Siersbaek-Nielsen

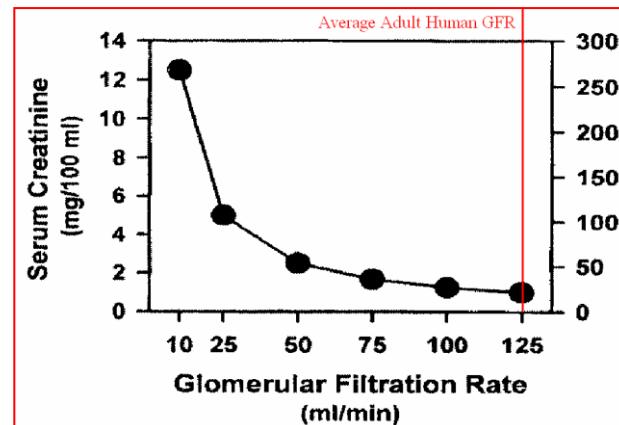
nomogram to estimate creatinine clearance from weight, age, and serum creatinine concentration (F9.9).

F9.9. Nomogram for rapid estimation of endogenous creatinine clearance. Lilly.



With a straightedge, join weight to age. Keep straightedge at crossing point of line marked "R." Then move the right-hand side of the straightedge to the appropriate serum creatinine value and read the patient's clearance from the left side of the nomogram.

When kidney function is stable, this gives a valid estimate. But in the presence of shock, severe cardiac failure, or oliguria, the estimate is too high.



F9.10. The relation between the most frequently used medical kidney parameters and the glomerular filtration rate.
Casarett & Doull.

9.5.2. Glomerular Filtration Rate

The Glomerular Filtration Rate, in ml/min, evaluates the volume of plasma being passed into Bowman's capsule. It is measured by administering a single injection of Inulin, a fructose polymer with molecular weight of 5200 Da. This sugar dissolves in the blood, but is not bound to any plasma component, is not metabolized or stored, is not reabsorbed or secreted. It becomes a permanent component of urine.

$$GFR \text{ (ml/min)} \approx \frac{[I_{Urine}](\text{mg/dL})}{[I_{Plasma}](\text{mg/dL})} \times \text{Urine Flow Rate (ml/min)}$$

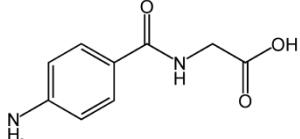
The numerator product is "mg excreted per minute", while the denominator is the concentration found in plasma from the injection. A normal GFR is about 125 ml/min. If this decreases, there is a possibility of longer half-lives for toxicants being eliminated, and consequently more toxicity. Any significant renal disease or toxicity will affect the GFR, as will hypotension or shock.

9.5.3. Tubular Secretion Rate

To evaluate the volume of plasma being passed through the kidneys as a whole, one can use, in the same way, PAH clearance.

P-Amino-Hippuric Acid is so well secreted into the urine by the tubules that about 91% of it is eliminated from the whole plasma present in the kidney in a single pass.

Normal values, compensated by 0.91, are 650 ml/min.



Dialysis machines remove toxins from the blood through diffusion, but are not specific and do not produce hormones and enzymes.

Membranes made of cellulose readily pass low molecular weight molecules (urea, creatinine, uric acid, electrolytes). Amino acids, vitamins, glucose and water are also lost, and so must be replenished.

A patient requires 3 sessions/week at 3-6 hours per session, during which 120 liters/week are filtered at a yearly cost of about 25,000 \$. By contrast, kidneys cleanse 1200 l/week.

As a result, urea levels in artificial dialysis patients are 14-200 mg/100 ml blood compared to 20-40 mg/100 ml of blood for normal individuals. Life expectancy in middle-age is only 5 years on artificial kidneys.

In the future, a chamber could be used to grow kidney cells from pigs, and a patient's blood made to interface with the

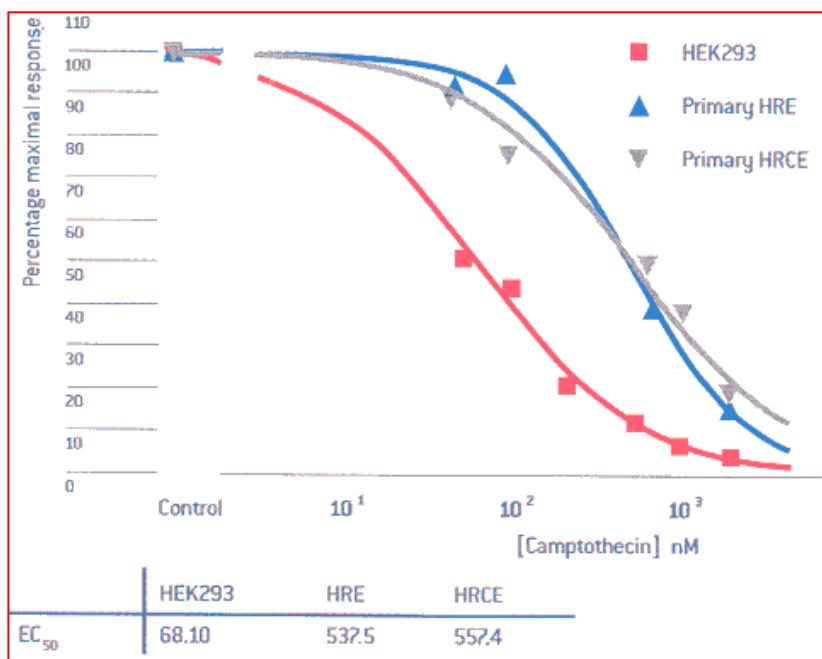
chamber. This system should provide improved control of blood ions, particularly potassium, in dialysis patients.

9.5.5. In Vitro

Initial research on the efficacy and toxicity of drugs is often conducted on cell lines that have been transformed, so that they may proliferate indefinitely, insuring repeatability of the test.

Specific cell lines are used to simulate the proximal tubule (LLC-PK₁, pig), and the distal tubule (MDCK, dog). Most of the current cell models cannot be used universally. Two problems are loss of renal characteristics and limited lifespan.

Renal continuous cell lines such as Human Embryonic Kidney 293 cells (HEK293) have been popular in pharmacology screening. The cells, assumed to be from a kidney fibroblast, endothelium or epithelium, were *transformed* in culture with adenovirus in the early 1970s. But a 2002 study suggested that HEK293 cells have many properties of immature neurons. The suitability of HEK293 as an *in vitro* model for kidney cell function was in question. When HEK293 was compared to Human Renal Cortical Epithelial Cells and Human Renal Epithelial Cells of more recent fabrication in their response to a drug, Camptothecin, responses were very different. Camptothecin induces apoptosis in a dose-dependent manner *in vitro*. As can be seen in the figure below, the effective concentration (EC₅₀) of Camptothecin on HEK293, compared to primary kidney cells in triggering apoptosis varies by a factor of almost 10. This probably led in the past to the use of lower and probably ineffective concentrations of drugs being used in tests of efficacy and toxicity.



F9.11. Effect of Camptothecin on viability of primary (HRE & HRCE) and continuous (HEK293) renal cells.

9.6. Short Kidney Cases

Modified from Sherman.

9.6.1. Kidney Case #1

James smoked cigarettes, beginning at age 17. He operated an antique car restoring business, handling a number of plastics, paints, and solvents: toluol, xylol, acetone, isopropyl alcohol, methylene chloride, ethylene glycol monoethyl ether, methoxyethyl acetate, acrylic paint systems, and a number of chemicals identified only as petroleum distillates, aromatic hydrocarbons, and aliphatic hydrocarbons.

In the MSDSs, many of the ingredients listed did not add up to 100%, others were not specific as to their identity, and others indicated only as solid acrylic, proprietary alcohol, bodying agent. Many of these chemicals are taken into the body not only via inhalation but through the intact skin. Many of them are eliminated in the urine, contacting kidneys and bladder cells.

James was 47 years old when he noticed blood in his urine. Test showed cancer of his right kidney, and it was removed in 1982. He stopped smoking cigarettes at the time his kidney cancer was diagnosed.

9.6.2. Kidney Case #2

Albert worked 24 years as a laborer at a company where lead was handled on a regular basis, for over 40% of his total lifetime. Lead is concentrated in the bones and excreted by the kidneys. Kidney cells are damaged, resulting in decreased ability to eliminate fluid and solute loads. While lead is stored in the bones, it interferes with the body's ability to make blood cells, producing anemia. Albert was admitted to the hospital because of kidney failure and hypertension when he was 58 years old. He had been admitted twice before because of lead poisoning, and within the previous month because of severe anemia. Six days later he was dead, his final diagnosis being chronic glomerulonephritis and uremia.

9.6.3. Kidney Case #3

David, a non-smoking police officer, was medically evaluated in January 1982. An Intravenous Pyelogram showed no abnormalities of his kidneys or bladder. In March 1984, David crashed into a utility pole in the automobile chase of a suspect. His patrol car and an electrical transformer exploded and burned. He was heavily exposed to the fumes and smoke. PCBs used as coolants in many older electrical transformers may be contaminated with dioxins (TCDD), potentially causing

malignancies. Even brief exposures have consequences, as PCBs have a long half-life within the body. By September 1984, he noticed blood in his urine. In January 1985 he had bladder cancer at 39 years old, and underwent surgical removal of the malignancy. Bladder cancer is rare at 39 years, and police duty does not involve exposure to toxic chemicals. With a baseline of no disease in 1982, exposure in 1984, and the development of a malignancy ten months later, a cause-and-effect relationship is highly likely. This sort of documentation is rare, in that few people have a body system evaluated twice within such a short time, with the appearance of cancer during the interval.

9.6.4. Kidney Case #4

Harry worked for 32 years buffing chrome-plated bumpers. He used a dry buffering compound and said that although it was extremely dusty work, he did not wear any sort of mask until after 1978. He said the bumpers were plated in an area about 100 m away from his workstation, and that the fumes and the acids "cut his wind", especially when equipment broke down. Exposure to chromium is associated with lung disease and kidney damage. Chromium compounds are taken into the body via inhalation, by ingestion with the saliva, and through the skin, causing damage at a number of sites. Chromium is excreted in the urine, and causes damage as it passes through the kidneys.

He first learned of high blood pressure and diabetes in 1967, and he took medication for each.

Harry is now 60 years old. His blood pressure readings are 250/130 on the right, and 240/142 on the left, and he has significant protein in his urine.

9.6.5. Kidney Case #5

Charles worked as a welder after finishing high school. He did aluminum welding, and then silver brazing for the next 16 years, followed by electric welding for the last 2 years. The brazing material's MSDS indicated: silver 50%, copper 15.5%, zinc oxide 16.5%, and cadmium oxide 18%. The product was "dangerous mostly from cadmium fumes. Inhalation of dusts or fumes of cadmium affects the respiratory tract and may affect the kidneys. Cadmium is listed by the National Toxicology Program as anticipated to be a carcinogen. Studies indicate that there is an increased incidence of prostatic cancer, and possibly kidney and respiratory cancer in cadmium workers."

The MSDS recommends respiratory protection. At 37 years old, Charles applied for life insurance. A routine examination found protein in his urine and kidney dysfunction. Biopsy revealed a focal sclerosing glomerulosclerosis, labeled as *nonspecific*. As an incidental finding, he had reported that he had essentially lost his sense of smell ten years previously.

9.7. Case Study: Acute Arsine Poisoning

(modified from Williams & Burson)

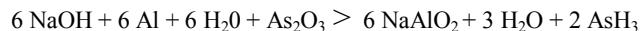
A patient was admitted with what seemed to be hemolysis. The hospital also reported that a fellow employee of the patient had become ill at about the same time and had been admitted to another hospital. A visit to the plant where both employees worked was organized. The concern was that other workers might have been exposed as well.

It was learned at the plant that the two ill employees had worked at cleaning a room on the preceding day. There had been a clogged floor drain in this room, and the workers used a drain cleaner that was one of the commercial products manufactured by the company. Once in the drain, the cleaner had bubbled and given off a gas with a pungent odor. Even though the employees developed headaches, they continued at their work for about four hours.

At home that night, the employees had become ill. They were nauseated, and vomited. One went to a hospital emergency room, was examined, and was sent home. The other went to another emergency room; his urine was sampled and it was bloody looking; he was admitted to the hospital. The first worker became

progressively worse and was admitted to a hospital the next day. In the meantime it was determined in the hospitals that both workers had severe hemolysis, which caused the bloody-looking urine. The only gas that will cause hemolysis is arsine. One of the necessary ingredients for the production of arsine is arsenic. The plant was therefore inspected for potential sources of arsenic, and samples from the floor drain were collected and analyzed. All the samples were analyzed by neutron activation analysis, which is somewhat expensive but is a relatively easy way to analyze multiple matrices. In addition, urine and blood from the two patients, the drain cleaner, and air samples were also submitted for analysis.

The plant consisted of a large work area and several offices. There were two loading docks, one small and the other large. One room was used for mixing the different products made there. In another area workers bagged products. The stopped-up floor drain from which the gases had bubbled was in the center of this area. The loading dock outside this room was higher than the floor of the room; when it rained, water on the loading dock would run into the building. The manager of the plant had worked there for only a few years and was unaware of any arsenic ever having been used in the plant. However, one of the maintenance men told investigators that before its present operation, the company had made arsenical herbicides, primarily arsenic trioxide; in fact, a tank located outside on the loading dock had previously been used for mixing arsenical herbicides. When the employees were conducting their general cleanup, they thought only water was in this tank and had drained it; the contents drained into the stopped-up drain. When the employees added their company's drain-cleaning product, primarily a mixture of sodium hydroxide, aluminum chips, and sodium, the gas had been emitted from the drain. Clearly the water and arsenic trioxide from the forgotten mixing tank had reacted to produce the gas arsine, as follows:



Arsine is extremely toxic. Arsine poisoning does not reveal itself in elevated arsenic levels in those patients who have been poisoned by arsine; it does not produce the same type of symptomatology as arsenic poisoning. Indeed, arsenic poisoning is entirely different from arsine poisoning.

The most important consideration was to demonstrate that arsenic was present in the drain, that an arsenic-to-arsine reaction could have taken place, and that there was arsine in the gas the employees had inhaled. Drain samples from the herbicide tank, samples from the drain cleaner, and the aluminum chips were all tested for arsenic. As shown in the Table, there was a great deal of arsenic, comparatively speaking, in the drain and in the herbicide tank. There was also some antimony. If antimony were substituted for arsenic in the reaction shown above, the result would be formation of stibene. However, stibene is not as stable as arsine, and it is highly unlikely that it would have caused the illness in the patients. The drain cleaner and the aluminum chips had no arsenic. Analysis of the urine from a number of other workers demonstrated that the two people who were ill had higher arsenic levels in their

urine than some of their fellow workers who had been in the vicinity of the drain but who had not gotten sick. A supervisor, who had never gone into the area where the drain was, showed no arsenic in his urine. It was concluded that the presence of arsenic in the drain resulted in the formation of arsine when the drain cleaner was added. The arsine gas caused the hemolysis in the patients.

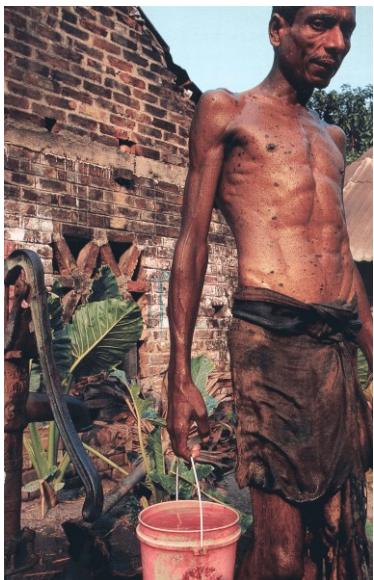
The two patients, after a very stormy medical course including renal failure and other problems, finally recovered, partly because such cases can now be treated with dialysis units. Before dialysis units were available patients with severe arsine poisoning usually died of renal failure. There are two effects of arsine poisoning on the kidneys. One is the breakdown of the red blood cells with excretion of hemoglobin, which will damage the kidneys; in addition, arsine itself may directly damage the kidneys. Both employees had to be dialyzed for several weeks.

T9.12. Arsenic and Antimony Levels in Environmental Samples Gathered at the Chemical Plant Where Arsine Poisoning Occurred		
Source	Concentration (ppm)	
	Arsenic	Antimony
Drain		
Upper fraction		
Liquid	440	44
Solid	970	55
Lower fraction		
Liquid	410	43
Solid	4,460	170
Air sample	13 μg	
Abandoned herbicide tank		
With agitation		
Before	7,490	393
After	11,400	698
Drain cleaner		
NaOH and NaNO ₃ solid	0.26	1.0
Aluminum chips	0.15	40.0

*Forty liters obtained on charcoal tubes minus background from unused tube

9.8. Bangladesh and Arsenic (National Geographic, 2002)

Because surface water had bacterial contamination, 12 million wells were dug in Bangladesh to minimize infection in the water supply.



Unfortunately, high levels of arsenic from the deeper wells has caused 7000 deaths since the early 1990s.

After 8-14 years, white or black spots (melanosis, see at left) appear on the skin (palms and soles), leading to gangrenous ulcers. Finally, renal diseases and cancers of lungs and bladder appear. It appears that arsenic attached to sedimentary grains is released in deep groundwater in great part by bacteria⁴.

More than 10 micrograms per liter of the poisonous element were associated with an elevated

risk of all-cause mortality^{6,7}.

The Environmental Protection Agency (EPA) signaled on October 31, 2001 that it will be reducing the amount of arsenic allowed in U.S. drinking water from a maximum of 50 micrograms per liter to the tighter 10 micrograms per liter.

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