Arsenic-Induced Carcinogenesis-Oxidative Stress as a Possible Mode of Action and Future Research Needs for More Biologically Based Risk Assessment

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Arsenic-Induced Carcinogenesis—Oxidative Stress as a Possible Mode of Action and Future Research Needs for More Biologically Based Risk Assessment

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Exposure to inorganic arsenic (iAs) induces cancer in human lungs, urinary bladder, skin, kidney, and liver, with the majority of deaths from lung and bladder cancer. To date, cancer risk assessments for iAs have not relied on mechanistic data, as we have lacked sufficient understanding of arsenic's pharmacokinetics and mode(s) of carcinogenic action (MOA). Furthermore, while there are vast amounts of toxicological data on iAs, relatively little of it has been collected using experimental designs that efficiently support development of biologically based dose−response (BBDR) models and subsequently risk assessment. This review outlines an efficient approach to the development of a BBDR model for iAs that would reduce uncertainties in its cancer risk assessment. This BBDR-based approach is illustrated by using oxidative stress as the carcinogenic MOA for iAs but would be generically applicable to other MOAs. Six major research needs that will facilitate BBDR model development for arsenic-induced cancer are (1) MOA research, which is needed to reduce the uncertainty in risk assessment; (2) development and integration of the pharmacodynamic component (MOA) of the BBDR model; (3) dose−response and extrapolation model selection; (4) the determination of internal human speciated arsenical concentrations to improve physiologically based pharmacokinetic (PBPK) models; (5) animal models of arsenic carcinogenesis; and (6) the determination of the low dose human relationship for death from cancer, particularly in lungs and urinary bladder. The major parts of the BBDR model are arsenic exposure, a physiologically based pharmacokinetic model, reactive species, antioxidant defenses, oxidative stress, cytotoxicity, growth factors, transcription factors, DNA damage, chromosome damage, cell proliferation, mutation accumulation, and cancer. The BBDR model will need to be developed concurrently with data collection so that model uncertainties can be identified and addressed through an iterative process of targeted additional research.

Contents

1. Introduction

Excessive exposure to arsenic has long been known to lead to human cancer of the skin and other internal organs including lung, urinary bladder, kidney, and liver (*1*). The noncancer health effects of arsenic include skin lesions (hypopigmentation, hyperpigmentation, and hyperkeratosis), cardiovascular disease, peripheral vascular disease, and diabetes (type II).

The ability of medicinally administered arsenicals to cause skin cancer was first noted by Hutchinson in 1887 (*2*). Later, in a 1968 ecological epidemiological study, Tseng et al. observed that skin tumor incidences were elevated in a concentration-dependent manner in a Southwest area of Taiwan with high arsenic exposures from underground wells (*3*). In the 1980s and 1990s, positive associations between elevated drinking water arsenic concentrations and internal cancers were found in Taiwan (*4, 5*), Chile (*6*), and Argentina (*7, 8*). These internal cancers had a higher degree of lethality than did arsenic-induced skin cancer.

In the United States, it is the carcinogenicity of arsenic that drives the risk assessments for arsenicals to low drinking water arsenic concentrations. Thus, the correct determination of the

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Table 1. Arsenic-Induced Cancer in Humans, Mice, and Rats: The Degree of Concordance

organ	human	mouse	rat
skin	yes	yes	
lung	yes	yes	
liver	yes	yes	yes
urinary bladder	yes		yes
kidney	yes		

For humans, the inorganic forms of arsenic are the only known carcinogens. In mice arsenite (in skin) (*10, 13*), MMA(III) (in skin) (*13*) and DMA(V) (in lung) (*11, 59*) have produced tumors. Gestational exposure to arsenite has produced liver tumors in mice (*14*). In rats, DMA(V) leads to urinary bladder tumors (*9*), while administration of TMAO has produced hepatocellular adenomas (*12*).

true population risk of arsenic-induced cancers to humans is of great importance. Risk assessors are confronted with unresolved biological questions such as determining the mechanism or mode of action (MOA) of arsenical-induced cancer and the dose−response relationship between low exposure levels and carcinogenicity. This is true despite many publications (over 3100 PubMed hits up to September of 2009) on arsenic and cancer. The current degree of concordance between human, mouse, and rat carcinogenesis by arsenic is shown in Table 1. For human exposures, inorganic arsenic (iAs) is the only known causal carcinogenic compound. In animal models, DMA(V)[1] exposures lead to carcinogenesis in rat bladder (*9*), transgenic mouse skin (*10*), and mouse lung (*11*). Trimethylarsine oxide (TMAO) has caused rat liver cancer (*12*). Arsenite, monomethylarsonous acid [MMA(III)], and dimethylarsinic acid [DMA(V)] have caused papillomas in transgenic mouse skin (*10, 13*). Gestational arsenite has caused a large number of tumors in mice including hepatic tumors (*14*).

Some view arsenicals as nongenotoxic carcinogens that can safely be regulated using threshold concepts; others think that the genotoxicity and clastogenicity of organic and inorganic arsenicals warrant linear extrapolation below the ranges where useful epidemiological or animal information is available. Currently, the linear default assumption is used by the U.S. EPA to determine the drinking water standard (maximum contaminant level, MCL) for arsenic.

In this review, six major areas for future research are presented that could substantially improve future risk assessments of arsenic. These six areas are connected to the use of biologically based dose−response model (BBDR) modeling and/or the selection of good extrapolation methods for the assessment of low-dose human cancer risk. This research and the application of the resulting BBDR model could replace the use of default assumptions in risk assessment. The results of such focused cell, animal, and human studies could accelerate the understanding and better regulation of environmental arsenicals.

An example of a BBDR model utilizing oxidative stress as a MOA of arsenical-induced carcinogenicity is presented. There are multiple possible MOAs for arsenic-induced carcinogenesis. A minireview article with binding of trivalent arsenicals to sulfhydryl groups as a MOA has been published elsewhere (*15*). These MOAs might act simultaneously or sequentially. This review will present just oxidative stress as a MOA. The model with oxidative stress as the MOA specifies exposure, a physiologically based pharmacokinetics (PBPK) model for arsenic to determine the internal tissue dose of the active, causal arsenical(s), and finally increasing numbers of mutations of genes sufficient to cause carcinogenesis. Such working pharmacokinetic−dynamic models would be refined and corrected by the results of future investigations of arsenic in biological systems. The majority of the remaining work to be done before such a model is useful is in the pharmacodynamic part of the model (hence the initial presentation of six areas for future needed research). We already have useable PBPK models for arsenic that were first developed in hamsters and rabbits (*16*) and then extended to humans (*17*). In addition, a PBPK model for arsenic in mice has been developed using iAs (*18*) or DMA(V) (*19*) as the administered compounds. There are still data needed to further develop a PBPK human model, particularly with respect to human interior tissue levels of speciated arsenicals, time course, and dose−response information. However, a recent human version of an arsenic PBPK model has been developed using primarily human information where possible (*20*). In conculsion, 50% of an overall BBDR for arsenic is already available; it is definitive information on the pharmacodynamic mode or modes of action that is (are) not currently available. Finally, it is hoped that this review can stimulate the effective planning and implementation of arsenic research programs and the acquisition of dose−response and time−course data sets that are necessary for the development of BBDR models for the estimation of true human carcinogenic risks from arsenic.

2. BBDR Models

A BBDR model typically consists of a pharmacokinetic component and a pharmacodynamic component. The pharmacokinetic component is often a PBPK model. The pharmacodynamic component is the MOA or the sequence of key biological events. For currently available BBDRs, the PBPK component is often more refined and better supported by data than is the MOA component. Examples of BBDR models for chemically induced adverse health effects include 5-fluorouracil and developmental toxicity (*21, 22*), dietary iodine and rat thyroid function (*23*), and formaldehyde-induced carcinogenesis (*24*).

2.1. MOA and Key Events in BBDR. In biological research, a mechanism of action is a detailed description of events from the initial cause of the exposure chemical to the final adverse biological effect. As an environmental regulatory agency, the U.S. EPA is often required to develop risk assessments and exposure standards before certainty or near certainty of biological evidence and data are available. Thus, in the U.S. EPA, the related concept of MOA has been developed. A MOA requires less detail and certainty than a mechanism of action, but a MOA should still be sufficiently detailed to be useful for its regulatory purpose. The EPA 2005 Cancer Guidelines include a fuller discussion of MOA and key events and are available from the Web site (http://www.epa.gov/raf/publications/pdfs/CANCER_GUIDELINES_FINAL_3-25-05.PDF). Several informative commentaries on the MOA concept, its human relevancy, and its use in environmental risk assessment have been published (*25*−*28*).

Thus, for cancer, a MOA is a sequence of key events and processes that starts with the initial interaction of an agent with a cell, proceeds through operational and anatomical changes, and finally results in cancer. Key events are quantifiable,

[1] Abbreviations: BBDR, biologically based dose−response model; DMA(V), dimethylarsinic acid; DMA(III), dimethylarsinous acid; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; MMA(III), monomethylarsonous acid; MOA, mode of action (A sequence of biological key events that result in an adverse effect. A MOA is less detailed than a full mechanism of action); PBPK, physiologically based pharmacokinetics; ROS, reactive oxygen species; RNS, reactive nitrogen species; TMAO, trimethylarsine oxide.

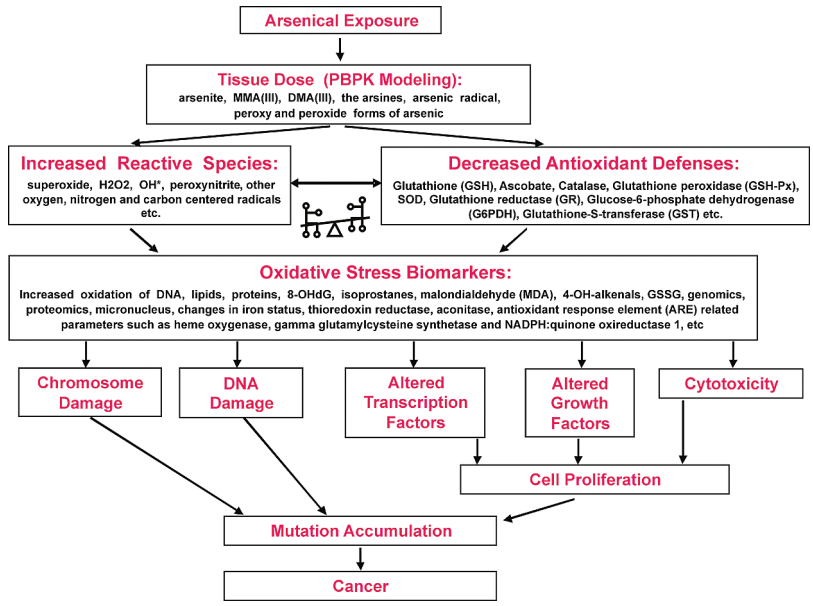

Figure 1. BBDR model for oxidative stress as a cause of arsenic-induced cancer. Many arsenic species as well as other carcinogenic mechanisms could be operating at the same time in different organs and species.

necessary precursor steps of the overall carcinogenic MOA that happen in a time-dependent and dose-dependent manner.

3. Six Research Needs for Arsenic Cancer Risk Assessment Via a BBDR

In the specific case of arsenic and risk assessment, there is a lot of published information available. However, almost all of the available information was never intended to be used by BBDR modelers, and so, it is often inapplicable, incomplete, or not germane enough to be useful for builders of BBDR models. Six important research needs for arsenic cancer risk assessment are given as follows.

3.1. MOA. Experimental work is needed to evaluate the relative contribution of genotoxic and nongenotoxic MOA(s) for arsenic in causing cancer. Better information on MOA can influence the extrapolation method (linear or nonlinear) selected for arsenic risk assessment.

It is important to determine the relative contribution of different species of arsenic to carcinogenicity and toxicity. The likely causes of arsenic carcinogenicity include the three compounds arsenite, MMA(III), and dimethylarsinous acid [DMA(III)] and to a lesser extent monomethylarsine, dimethylarsine, and trimethylarsine. Little is known about these latter three highly reduced methylated trivalent arsines. We do not know if these highly DNA-reactive arsines (*29*) occur in vivo to a biologically important extent.

The MOA is the single most important unknown in arsenic risk assessment. Either linear (for a genotoxic MOA) or nonlinear (for a nongenotoxic MOA) extrapolation techniques could be used for arsenic. A BBDR model can use both MOAs (genotoxic and nongenotoxic) sequentially or simultaneously as has been done for formaldehyde (*24*). The MOA question of genotoxic versus nongenotoxic for arsenic has orders of magnitude impact on what the cancer risks of arsenic exposures actually are.

The formaldehyde BBDR cancer model had to grapple with the issues of genotoxic and nongenotoxic causes of cancer. It is expected that a BBDR model for arsenic would be much more complicated than the model for formaldehyde. This is because of the multiplicity of MOAs that could be involved in arsenic-induced cancer. Thus, the use of more scientific information in assessing risk (via MOA, BBDR, or any other means) will help meet current and future societal goals and regulatory agency responsibilities. Under the 1996 Safe Drinking Water Act amendment, the U.S. EPA must reevaluate the arsenic drinking water standard every 6 years.

3.2. MOA in BBDR. Development of BBDR models is needed to integrate data on pharmacokinetic and pharmacodynamic behavior of arsenicals in a coherent framework for evaluation of the health effects and risks of specific arsenical exposures. Although we already have several usable published PBPK models for arsenic (*16*−*20*), more work is needed on the development of the pharmacodynamic (MOA) part of the overall BBDR model. To develop a BBDR model for arsenic, we need complete data sets with both time−course and dose−response information on the components of a MOA (key events). A key consideration is the level of detail that actually is needed in the description of the MOA. Do we need as much or more detail than is presented in Figure 1 or is a simpler BBDR good enough?

3.3. Dose−Response and Extrapolation Model Selection. It is challenging to interpret typical chronic animal exposures to iAs, because one is never sure if the biological effects being observed are due to the administered iAs itself (e.g., arsenite) or to methylated metabolites such as MMA(III) and DMA(III).

Dose−response and time−course data in experimental animals and cellular systems should be obtained that assist in selecting low-dose extrapolation models for arsenic exposures below those in which useful information is available from current human epidemiological and experimental animal studies. Several biological systems could be of particular use for such studies. Transgenic mice lacking the enzyme arsenic (+3 oxidation state) methyltransferase (As3MT) (*30*) could be used to obtain biological information after exposure to arsenite or arsenate, both nonmethylated species of arsenic. Methylation of arsenic is a major pathway of arsenic metabolism and excretion in most mammals (*31*). The diminished methylation of arsenicals observed in these transgenic mice (*30*) will provide data where it is easier to link the observed biological effect with a single causal arsenical (iAs). Exposures to monomethylated

and dimethylated arsenicals in As3MT transgenic mice are also important because further methylation of the administered arsenicals will be greatly diminished. Mice are particularly useful in arsenic research because such a substantial PBPK model has been developed for the mouse (*18, 19*) and no similar PBPK model exists for rats. Rats sequester large amounts of DMA(III) in their red blood cells, and this sequestering requires an additional pharmacokinetic compartment. Similarly, guinea pigs could be used as experimental animals because they do not methylate arsenic (*32*). Alternatively, cells could be selected with minimal or zero capacity to methylate arsenic (*33*).

3.4. Internal Human Speciated Arsenical Concentrations. Despite the availability of some internal human tissue arsenic concentrations, there is a big data gap on speciated arsenical levels in the target human tissues of carcinogenic concern (lungs, urinary bladder, kidney, liver, and skin). There are hundreds or thousands of determinations of arsenic levels in drinking water, food, blood, and urine. However, these are not highly useful to develop and refine the PK part of a human PBPK model. Blood is cleared of many arsenicals so fast that it is often thought of as a poor indicator of tissue arsenical levels. For example, after intravenous administration of DMA(V) to female B6C3F1 mice, the first and second half-lives of distribution and elimination were 7.7 and 43.3 min, respectively (*34*).

Because of the limited utility of blood and urine arsenic concentrations, nearly any interior human organ might provide useful data for model developers. Attractive research opportunities are human placenta, cancer resections, and biopsies. When internal human tissue samples are available, it is desirable to quantify the urinary output of arsenic, drinking water, and possibly food intake of arsenicals so that the most complete information is available to accompany the interior human tissue arsenical(s) concentrations.

3.5. Animal Models of Arsenic Carcinogenesis. MOA, time−course, and dose−response data are needed in the three most studied animal models of arsenic carcinogenesis—mouse skin, mouse lung, and rat urinary bladder. Future studies could include (1) cancer bioassays of DMA(III) and TMAO in mouse skin, (2) gestational and lifestage arsenical exposures for mouse lung, (3) gene expression and cell proliferation studies in rat bladder, and (4) biomarker research. A major advantage of laboratory animal or cellular studies is that the internal dose can be determined, whereas this internal organ dosimetry information is normally lacking in epidemiological studies.

3.6. Low-Dose Relationship for Human Cancer. Research is needed to determine the low-dose relationship for cancer end points in humans for bladder, lung, and other internal organs that can often lead to human death. This can be done for skin cancer as well, although only a low number of arsenic-induced skin tumors actually result in death. To actually perform research and obtain meaningful, useful data in the low-dose arsenic-induced cancer epidemiology area are an extremely difficult undertaking. At present, the two best data sets for linking drinking water arsenic and human tumors come from Taiwan (lowest exposure group of 0−300 ppb) (*4*) and Chile (a single exposure group of about 420 ppb) (*6*).

The most attractive areas to do this work would include Bangladesh and India for current arsenic exposures and Chile and Taiwan for past arsenic exposures. High-concentration hazard identification studies are not needed anymore. Human health effects research with low environmental arsenic concentrations (e.g., below 300 ppb) is an area that can help us move forward.

4. An Oxidative Stress BBDR Model for Arsenic's Carcinogenicity

In a 2001 review article (*35*), nine possible MOAs for arsenic carcinogenesis were presented: chromosome abnormalities, oxidative stress, altered growth factors, cell proliferation, promotion and/or progression in carcinogenesis, altered DNA repair, p53 gene suppression, altered DNA methylation patterns, and gene amplification. The early biological key events in carcinogenic MOAs are likely to be (1) binding of trivalent arsenicals to tissue targets such as sulfhydryl groups and/or selenium atoms (*15*); (2) formation of superoxide, hydrogen peroxide, and other reactive oxygen species (ROS) (*36, 37*); and/or (3) hypomethylation of DNA (*38*). Recently, there seems to be an emerging consensus that the likely carcinogenic MOAs for arsenic depend on these three biochemical key events. It is not clear which arsenic species and key events are most important in the five organs in which arsenic is carcinogenic. Mechanisms of arsenic carcinogeneis in lung (in humans or mice), urinary bladder (in humans or rats), and skin (in humans and normal and transgenic mice) may be quite different, particularly with respect to factors such as the concentrations of oxygen, speciated arsenicals in tissues (*39*), endogenous reducing agents, and ferritin (*40*). Lungs are exposed to the highest oxygen tensions in the body; thus, ROS could preferentially form in pulmonary tissues. Urinary bladder is exposed to arsenicals both by normal blood circulation and from the lumen of the bladder (*41*) during excretion of inorganic and methylated arsenicals, particularly DMA(V) and DMA(III). Skin is exposed to normal amounts of oxygen and circulating arsenicals but is unusually sensitive to both carcinogenic and noncarcinogenic effects of arsenic hypopigmentation, hyperpigmentation, and hyperkerotosis.

In the final section of this review, a plausible MOA for arsenic causing cancer by oxidative stress will be presented. Oxidative stress is presently one of the most popularly proposed MOA for arsenic-induced cancer. The topic of oxidative stress and arsenic carcinogenesis has been reviewed a number of times (*36, 37, 42, 43*).

DNA methylation changes are also an interesting possible MOA of arsenic carcinogenesis. This could proceed via methylation changes in either tumor suppressor gene or oncogenes (e.g., c-myc, rac1, p16, and cyclin D1, etc.). However, even if the DNA methylation MOA of arsenic carcinogenesis is true, we do not really know what the important target genes are. Alternatively, DNA methylation changes could act via more numerous nononcogenes such as the estrogen receptor α in gestational carcinogenesis mouse models, as suggested by the Waalkes group (*38*).

4.1. Tissue Dose. As an example of the type of dose−response tissue levels of speciated arsenicals that could be included in a BBDR, the iAs (which includes both arsenite and arsenate) and total of all found arsenic species tissue concentrations found in a 30 day drinking water exposure to arsenite mouse study are presented in Table 2 (*44*).

Mice are able to methylate and excrete large amounts of administered arsenicals. Using a purified diet (AIN-93M) to reduce food arsenicals lowered the mouse tissue arsenic background levels. Therefore, the effects of low arsenite concentrations in the drinking water could be seen. The arsenic tissue levels were so low in control animals that they were not detectable in two instances and only 2.4 ng/g total arsenic species in lungs. In three comparisons, the lowest drinking water arsenite concentration (only 0.087 ppm expressed as arsenite or 0.05 ppm expressed as elemental arsenic (the former

Table 2. Mouse Liver and Lung Concentrations of Arsenicals after 30 Days of Exposure to a Wide Range of Drinking Water Arsenite Concentrations[a]

arsenite drinking water concentration (ppm)	iAs in lungs (ng/g)[a]	total As in lungs (ng/g)[a]	iAs in liver (ng/g)[a]	total As in liver (ng/g)[a]
0 (controls)	not detected[b]	1.38 ± 0.5	not detected[b]	17.0 ± 5.8
0.087	21.1 ± 2.0[c]	22.5 ± 2.4[c]	17.1 ± 3.0[c]	20.7 ± 2.0
0.434	26.2 ± 3.2[c]	30.1 ± 3.3[c]	19.8 ± 0.8[c]	21.1 ± 0.7
1	22.4 ± 1.8[c]	50.9 ± 6.9[c]	16.8 ± 1.4[c]	21.2 ± 1.9
10	16.8 ± 3.0[c]	105 ± 14.5[c]	27.2 ± 1.6[c]	74.0 ± 8.6[c]
85	2.0 ± 2.0	316 ± 51.3[c]	259 ± 53[c]	658 ± 138[c]
150	73.5 ± 18.8[c]	1128 ± 133[c]	615 ± 112[c]	943 ± 130[c]
250	242 ± 29.1[c]	2345 ± 133[c]	1075 ± 192[c]	2033 ± 358[c]

[a] Values are the means ± SEMs for five or more samples. The experimental animals were adult female C3H mice. Data are expressed as ng of the arsenical [e.g., arsenate, MMA(V), and DMA(V)] and not as elemental arsenic. The total As is the sum of arsenate, MMA(V), and DMA(V). To decrease their background tissue arsenical concentrations, mice were fed a purified diet (AIN 93M) for 14 days prior to the study and also during the 30 days of drinking water arsenite exposure. [b] The limit of detection for iAs is about 0.2 ng/g tissue. Arsenic drinking water concentrations are expressed as ppm sodium arsenite and not as elemental arsenic. [c] Statistically significant vs control at $P < 0.001$ via a Kruskal−Wallis one-way analysis of variance on ranks followed by individual t tests to determine P values. This data is from a study which has been published as an abstract (*44*).

American MCL)) significantly ($P < 0.001$) increased tissue arsenic concentrations up to 17−23 ng/g.

Substantial tissue arsenical concentrations seem to build up in the 10 ppm arsenite and over a drinking water range. In the 10 ppm arsenite treatment group, the total mouse lung and liver arsenic concentrations were 105 and 74 ng/g (Table 2) (*44*). When examined either arithmetically or with both the *X*- and the *Y*-axis logged, the total arsenic levels fit both a straight line ($y = a + bx$) and a simple power equation ($y = a + bx^c$) fairly well (all r^2 values were between 0.78 and 0.99).

The study of Kenyon et al. (*39*) is another recent pharmacokinetic study of iAs (arsenate) in mice. Female C57Bl/6 mice were exposed for 12 weeks to 0.5, 2.0, 10, and 50 ppm of arsenate (expressed as elemental arsenic) in their drinking water. Speciated arsenic concentration information is available on the drinking water, oral arsenic input to the mice, 24 h urinary output, and six organs (blood, lung, urinary bladder, kidney, liver, and skin) (*39*). This study clearly shows that urinary arsenic metabolite profiles are not necessarily reflective of target tissue dosimetry and that there is substantial selected accumulation of monomethylated arsenic species in the mouse kidney and dimethylated arsenic species in the mouse lungs.

4.2. Increased Reactive Species and Decreased Antioxidant Defenses. Figure 1 presents oxidative stress as a possible MOA for arsenic carcinogenesis. The first part of the model is simply the arsenic exposure and tissue arsenic levels (PBPK). The next components of the overall BBDR model are connected to the increase in the concentrations of reactive (oxidizing) species {free radicals centered in arsenic atoms, oxygen atoms (ROS), nitrogen atoms [reactive nitrogen species (RNS)], carbon atoms, and so on} and the decrease in cellular antioxidant defenses against oxidative attack. Exactly how superoxide concentrations are raised by various arsenical(s) remains an unsolved mystery. Increased superoxide concentration and its downstream consequences such as hydrogen peroxide, peroxynitrite, hydroxyl radical, etc. seem particularly important in mediating arsenic's effects. An experimental time−course study showing decreased antioxidant defenses is presented in Table 3 (e.g., decreases in GSH, glucose-6-phosphate dehydrogenase, GSH reductase, catalase, and GSH peroxidase) (*45*).

Glutathione, as a sulfhydryl-containing tripeptide, interacts with trivalent arsenicals both as an antioxidant and as a chelator. Complexes between reduced glutathionine and trivalent arsenicals are known to exist by the criteria of both theory and experiment (*46*). Many of the parameters shown in the antioxidant defenses box of Table 3 (reduced glutathione, oxidized glutathione, ascorbate, catalase, glutathione peroxidase, superoxide dismutatse, glutathione reductase, glucose-6-phosphate dehydrogenase, and glutathione-*S*-transferase) are quantifiable parameters of the biological effects of arsenic. It is much easier to measure the concentrations of these antioxidant defenses than to either directly or indirectly determine free radical concentrations.

Table 3. Time Course of Arsenic-Induced Mouse Hepatic Oxidative Stress and Toxicity[a]

Endpoint	Time in months				
	3	6	9	12	15
Malondialdehyde (MDA via TBARS)			↑	↑	↑
Glutathione (GSH)		↓	↓	↓	↓
Glucose-6-phosphate dehydrogenase (G6PDH)		↓	↓	↓	↓
Glutathione reductase (GR)				↓	↓
Catalase				↓	↓
Glutathione peroxidase (GSH-Px)			↓	↓	↓
Glutathione-S-transferase (GST)	↑	↓	↓	↓	↓
Alanine aminotransferase (ALT)				↑	↑
Aspartate aminotransferase (AST)				↑	↑
Alkaline phosphatase (ALP)					↑
Na^+/K^+ ATPase			↓	↓	↓
Albumin					↓
Liver Weight				↑	↑
Liver Histology				Fatty infiltration	Fatty infiltration, Fibrosis

[a] The livers of mice exposed to 3.2 ppm iAs in their drinking water developed oxidative stress over time (*45*). Arrows refer to statistically significant increases (in red) or decreases (in green). Reprinted with permission from ref *61*. Copyright 2010 Wiley-Blackwell.

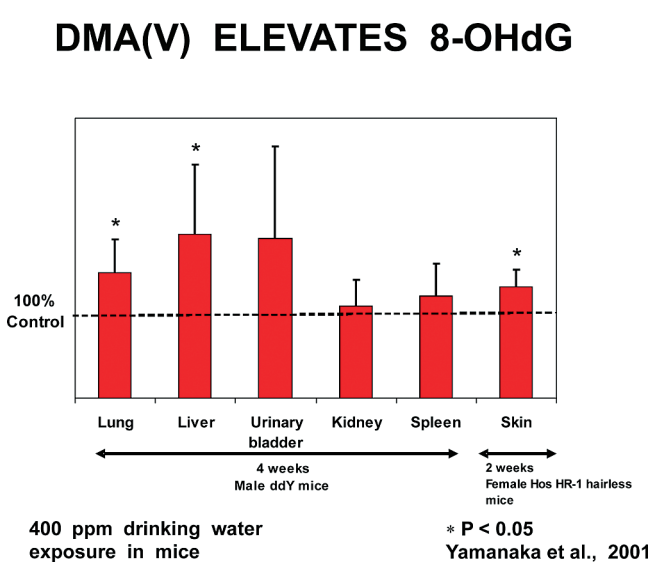

Figure 2. Administration of DMA(V) to mice for 2−4 weeks increases the concentration of 8-OHdG in DNA. In some cases, the degree of DNA oxidation reaches statistical significance (liver, lung, and skin), and in others cases, it does not reach the $P < 0.05$ level (*47*). Reprinted with permission from ref *61*. Copyright 2010 Wiley-Blackwell.

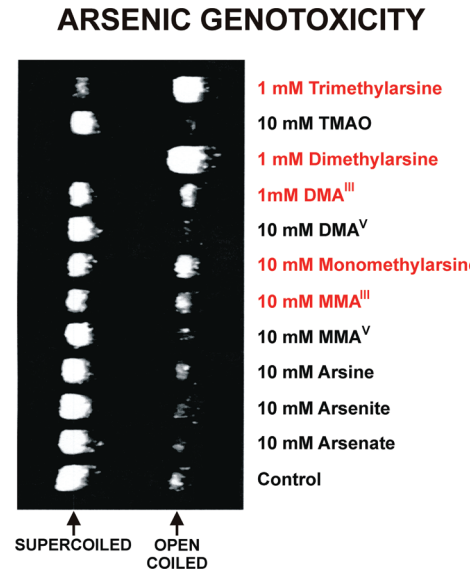

Figure 3. In vitro effect of 11 arsenicals on pBr 322 plasmid supercoiled DNA. All of the common arsenicals in the linear "Challenger" metabolism pathway (*60*) from arsenate to TMAO are included. Four additional arsines are included as well. Generally, the most active arsenicals [trimethylarsine, dimethylarsine, monomethylarsine, DMA-(III), and MMA(III)] were trivalent and had a high degree of methylation and often possessed hydrogen atoms rather than hydroxyls (*29*). Reprinted with permission from ref *29*. Copyright 2003 American Chemical Society.

4.3. Oxidative Stress Biomarkers. The large central box in Figure 1 is labeled "oxidative stress". Figure 2 shows data for a classical oxidative stress parameter, increased 8-hydroxy-2′-deoxyguanosine (8-OHdG) concentration, in mouse tissues after exposure to DMA(V) (*47*). In all six cases, DMA(V) increased, at least numerically, the mouse tissue 8-OHdG concentrations. In three cases (lung, liver, and skin), the increases in 8-OHdG concentrations were statistically significant. Many other common indicators or biomarkers of oxidative stress are shown in Table 3. Among the most important are the oxidation of DNA (usually 8-OHdG), malondialdehyde, 4-hydroxynonenal, thioredoxin reductase, and ARE-related parameters. Changes in these parameters are evidence that oxidative stress really is occurring in the tissues, species, or cells being studied. From the sustained oxidative stress resulting from continued elevated cellular arsenic and ROS concentrations, many important biological effects of oxidative stress are the next of the causal dominos to fall in the carcinogenic pathway(s).

4.4. DNA Damage. At the next level of Figure 1, the five items listed are chromosomal damage, DNA damage, altered transcription factors, altered growth factors, and cytotoxicity. DNA damage can certainly be a key event in carcinogenesis. Arsenic has long been known to damage chromosomes, although it is a poor point mutagen (*48*). Clastogenesis could also be a way for arsenic to induce genotoxic effects in carcinogenesis. Experimentally, the ability of some arsenicals to cause DNA damage in vitro (DNA strand breaks) is shown in Figure 3. In this study by Andrewes et al. (*29*), it is the more methylated trivalent (and thus oxidizable) arsenicals that have a high degree of DNA damaging potency. Various experiments with antioxidants have suggested that such DNA damage is mediated via oxidative stress (*37, 40, 49*).

4.5. DNA Repair, Cell Proliferation, and Mutation Accumulation. DNA repair has both a high capacity and a high fidelity (*50, 51*). It consists of many systems including base excise repair/single strand break repair, nucleotide excision repair, mismatch repair, double strand break repair, and cross-link repair (*52*). The fact that 30−40% of humans develop an invasive tumor during their lifetimes demonstrates that all of the biological processes that defend us against carcinogenesis (including all of the forms of DNA repair, cell cycle checkpoints, p53 systems, apoptosis, and the immune system) are not 100% effective. Inhibited or incorrect DNA repair has long been a favorite component of the MOA of arsenic as a carcinogen (*35, 53*). On the basis of a study of six chemical forms of arsenic and the genetoxic parameters of chromosome aberrations, sister chromatid exchanges (SCE), mutagenicity in L5178Y/Tk(±) mouse lymphoma cells, *Salmonella* reversion mutation assay, and prophage induction in *Escherichia coli*, the authors concluded that some arsenicals were potent clastogens but all were very low in the ability to induce point mutations (*48*).

In genomic studies of arsenicals, some of the gene transcripts that arsenic exposures often affect that could be causal in carcinogenic processes include c-myc, ras superfamily member rac1, p16, and cyclin D1. c-Myc is a transcription factor that binds to enhancer box DNA sequences and recruits histone acetyltransferases to DNA. Myc is upregulated in many cancers and regulates cell growth, apoptosis, differentiation, and stem cell self-renewal.

The Ras superfamily of genes codes for small GTPases and includes rac1 (Ras-related C3 botulinum toxin substrate 1). Rac1 is a member of the Rac subfamily of the Rho family of GTPases. Rac1 regulates cell cycle, cell−cell adhesion, motility, and epithelial differentiation.

p16 is a tumor suppressor protein, which regulates the G1 phase of the cell cycle. p16 interacts with the tumor suppressor protein p53 and its protein sequester MDM2. The p16 gene is frequently mutated or deleted in a wide variety of tumors. Cyclin D1 is a member of the cyclin family of proteins that function as the regulatory subunits of cyclin/cyclin-dependent kinase enzymes that regulate entry into and progression through the cell cycle. Overexpression of cyclin D1 has been found in many cancers including breast, prostate, and colon cancer and lymphoma.

Recent genetic studies have shown that the average number of genetic mutations was 63 in pancreatic tumors (*54*) and 60 in human glioblastoma multiforme (*55*). Not all of these mutations are strong causes of carcinogenesis; many of the

observed mutations are secondary effects of the carcinogenesis process. For the studied human glioblastoma multiforme and pancreatic cancers, point mutations were the most common finding although amplifications and deletions also occurred. This genetic information is germane to the number of causal mutations that a chemical carcinogen such as arsenic might be causing.

The stimulation of cell proliferation by either positive mitotic stimulation, necrosis-induced regenerative hyperplasia, or altered apoptosis could drive carcinogenesis via oxidative stress. Arsenic-induced changes in growth and transcription factors could similarly drive higher rates of cell proliferation. Eventually, the BBDR proposed in Figure 1 proceeds to either cell proliferation or the accumulation of mutations as the important drivers of arsenic's carcinogenesis. For either of these cases, Moolgavkar type two stage clonal growth models (*24, 56, 57*) can be used for the final stages of a BBDR.

5. Conclusion

The carcinogenicity of arsenic to humans was first noted in 1887 for skin and confirmed in 1968 for skin (*3*) and the 1980−1990s for urinary bladder, lungs, liver, and kidney (*4*−*8*). Despite this long awareness of the carcinogenicity of arsenic, determining the MOA of arsenic-induced carcinogenesis has been difficult. Drinking water MCLs are determined by many factors including anticipated health benefits from reduced exposure and implementation costs. This 10 ppb water standard is used by many developed countries and was chosen by the World Health Organization in 1993.

Recently, the U.S. EPA implemented a lowered MCL for arsenic (50 to 10 ppb) in January of 2006. Different governmental authorities have chosen either lower or higher drinking water arsenic standards (e.g., New Jersey, 5 ppb; Australia, 7 ppb; many countries, including the United States, 10 ppb; India, 50 ppb; and Bangladesh, 50 ppb). The People's Republic of China uses an arsenic drinking water standard of 50 ppb for small water supplies in rural areas and 10 ppb for large water supplies in cities.

Several attempts with various degrees of sophistication and completeness have already been published on arsenic BBDR models for cancer risk estimation (*41, 58*). Clewell et al. (*41*) is a progress report on an ongoing project and does not present a plan for a complete BBDR, although they do propose a margin of exposure approach motivated by mechanistic considerations. The Ling and Liao study completed a risk assessment for eating tilapia containing high levels of arsenic because the fish were grown in arsenic-contaminated water (*58*). This study addresses the specific risk from arsenic-contaminated farmed fish and is not centered on the broader environmental issues of health risks caused by arsenic in drinking water, other general food sources, and air.

It is perhaps because arsenic does so many things (e.g., binds to heteroatoms of sulfur, selenium, and molybdenum; causes oxidative stress; and changes the methylation states of DNA, etc.) that agreement on probable MOA(s) for arsenic has been so slow to emerge. From looking at the MOA-containing BBDR model presented in Figure 1, one immediately realizes how important targeted research data are to provide an experimental basis with which to build quality mathematical models.

At present, we have usable animal models of carcinogenesis for most all of the organs in which arsenic causes cancer (Table 1). PBPK models of arsenic already exist for both the mouse (*18*) and the human (*20*). We also have usable proposed MOA(s) and BBDR models (Figure 1 and ref *15*) that are good for generating testable hypotheses about arsenic-induced cancer. Performing the type of research described in the six research needs areas will help provide important needed data to future builders of BBDR models and future risk assessors of arsenic-induced cancer. This review has presented several data sets that are components of the overall arsenic BBDR presented as Figure 1 as examples of data that could be useful to BBDR modelers in the different sections of the arsenic BBDR model: (1) tissue dose, iAs, and total arsenic concentrations in mouse lung and liver in Table 2; (2) decreased antioxidant defenses, six different antioxidant parameters in a chronic mouse study in Table 3; (3) oxidative stress, elevated 8-OHdG concentrations in six mouse organs in Figure 2; and (4) DNA damage by five different arsenicals in Figure 3.

BBDR models perform best when they are built from extensive dose- and time-dependent data sets of key events (the intermediate steps along the pathways of carcinogenesis). To date, it has been difficult to organize and execute the type of multiple disciplinary and multiparameter studies of arsenic's biological effects that could help determine what the major versus minor pathways of arsenic-induced carcinogenesis are. We are not suffering from a shortage of arsenic data per se but from a shortage of useful dose- and time-dependent data sets of important intermediate events of carcinogenesis. Hopefully, in the future, better planned and executed MOA-related studies with both dose−response and time−course components that are useful in the construction of BBDR models will be done. BBDR models can improve on the present day use of default assumptions in the cancer risk assessment of arsenic.

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