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Invited Review

CARBOFURAN TOXICITY

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Carbofuran, an anticholinesterase carbamate, is commonly used as an insecticide, nematicide, and acaricide in agricultural practice throughout the world. Due to its widespread use in agriculture, contamination of food, water, and air has become imminent, and consequently adverse health effects are inevitable in humans, animals, wildlife, and fish. Currently, carbofuran's involvement is most frequently encountered in malicious poisoning. The literature on chemical properties, acute toxicity data, poisoning incidences, pharmacokinetics, and mechanism of toxicity of carbofuran is briefly reviewed. Much emphasis is given to the metabolism of carbofuran, and the impact of carbofuran and its two major metabolites (3-hydroxycarbofuran and 3-ketocarbofuran) on overall toxicity. Biochemical (cholinergic and noncholinergic), hematological, and immunological effects induced by carbofuran are discussed in detail. Carbofuran and/or its major metabolites can cross the placental barrier and produce serious effects on the maternal–placental–fetal unit. Carbofuran's toxicity can be potentiated by simultaneous exposure with other cholinesterase inhibitors. Literature on various biomarkers of carbofuran exposure and on induced adverse health effects is also presented. To date, a combination of atropine and memantine remains the most effective antidotal treatment against acute carbofuran toxicity.

Currently, among all classes of pesticides, carbamates are most commonly used because among the alternatives, organochlorines have a long-lasting residue persistence problem, and organophosphates (OPs) are extremely toxic and pose a delayed neurotoxicity problem. Among carbamates, carbofuran (Furadan) is most commonly used in agriculture and forestry as a broad-spectrum systemic insecticide, nematicide, and acaricide. As a result of its widespread use, air, food, and surface water and groundwater are contaminated with carbofuran and its metabolites (Hallberg, 1987; Bushway et al., 1992; Kross et al., 1992; Waite et al., 1992), which may affect the health of everyone. Carbofuran has high toxicity to humans through the oral and inhalation routes of exposure, and therefore may pose a serious threat to those who are in immediate contact (in manufacturing and formulating plants and in crop fields).

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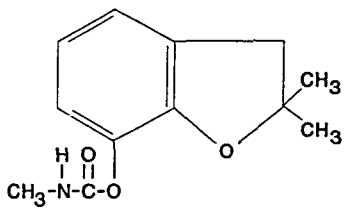
Several investigators have reported accidental carbofuran poisoning in humans, pets and domestic animals, and fish and wildlife. Currently, its involvement is most frequently encountered in malicious poisonings, especially in pets and birds. Based on available acute toxicity data, carbofuran is found to be an extremely toxic chemical. In recent years, abundant literature has become available focusing on specific aspects of carbofuran toxicity. This review provides comprehensive information on carbofuran toxicity in mammals.

CHEMICAL PROPERTIES

Carbofuran, commonly marketed as Furadan, has several other synonyms: Bay 70143, curaterr (Bayer AG), D 1221, ENT 27164, FMC 10242, NIA 10242, Pillarfuran, and Yaltox. It is chemically known as 2,3-dihydro-2,2-dimethyl-7-benzofuranol methylcarbamate, or 2,2-dimethyl-2,3-dihydro-7-benzofuranyl-*N*-methylcarbamate, or methyl carbamic acid 2,3-dihydro 2,2-dimethyl-7-benzofuranyl ester, or 2,2-dimethyl-7-coumaranyl-*N*-methyl-carbamate. The chemical structure of carbofuran is shown in Figure 1. The chemical formula is $C_{12}H_{15}NO_3$ and the molecular weight is 221.26. Other chemical properties include white, crystalline solid, melting point 150–152°C, specific gravity 1.18 at 20°C/20°C, vapor pressure 2×10^{-5} mm Hg at 33°C, and water solubility 700 ppm at 25°C.

POISONING INCIDENCES

In the past two decades, the use of carbofuran in agricultural practice has increased enormously. As a result of its widespread use and high toxicity, many incidences of accidental poisoning in humans, animals, birds, and fish are reported worldwide. In a case report, Coleman et al. (1990) described an occurrence of carbofuran poisoning in three female farm workers in Jamaica. Colvin (1987) described poisoning incidences in Georgia, during 1980–1985, involving domestic animals. These incidences were related to poor management practice and accidental contamination of feed with carbofuran. In Canada, Schuh and Blakley (1988) reported carbofuran poisoning in cattle



CARBOFURAN (MOL. WT. 221.26)

FIGURE 1. Chemical structure of carbofuran.

TABLE 1. Some Incidences of Carbofuran Poisoning/Exposure in Humans, Animals, and Birds

Species	Country	Reference
Human	Jamaica	Coleman et al. (1990)
	Canada	Hussain et al. (1990)
	United States	Zwiener and Ginsburg (1988)
		Draper et al. (1981)
	Philippines	Doevinsohn (1987)
Cat	Canada	Smith and Lewis (1988)
Dog	Canada	Smith and Lewis (1988)
Cattle	Canada	Smith and Lewis (1988)
		Schuh and Blakley (1988)
	United States	Miller and Corselius (1986)
		Osheim et al. (1985)
		Gardner (1976)
Sheep	Bulgaria	Topalski et al. (1987)
	Romania	Suteanu et al. (1986)
Pig	United States	Colvin (1987)
Birds	Canada	Smith and Lewis (1988)

consuming canola seeds treated with carbofuran. Some other selected incidences of carbofuran poisoning are listed in Table 1. Currently involvement of carbofuran is most frequently encountered in malicious poisonings, especially in dogs, cats, and birds.

TOXICITY DATA

General acute toxicity data of carbofuran in different species are summarized in Table 2. Overall, carbofuran has high toxicity to mammals through the oral and inhalation routes of exposure but has low toxicity through the dermal route. For details about adverse health effects of carbofuran on humans and animals following short- and long-term exposure, see the recently published review (Anonymous, 1988).

CLINICAL MANIFESTATIONS

Experimental laboratory animals, following oral or parenteral exposure to carbofuran, exhibit the onset of toxic signs such as salivation, chewing, and fine tremors within 5–15 min. With frequent propensity, signs of maximal severity, including muscle fasciculations and convulsions, are evident from 15 min to 1 h and persist for about 2 h. Thereafter, toxic signs are seen for up to 3 h with reduced severity. In general, the toxic manifestations are characteristic of anticholinesterase with parasympathetic preponderance exhibiting profuse salivation, lacrimation, miosis, hypothermia, muscle twitch and fasciculations, body tremors, and convulsions. Overall, carbofuran elicits toxic

TABLE 2. Acute Toxicity Data of Carbofuran in Different Species

Species	Route	Toxicity parameter	Dose	Reference
Rat	Oral	LD50	5.0 mg/kg	Matsumura (1985)
	SC	Lethal	2.5 mg/kg	Gupta and Kadel (1989a)
	IP	Lethal	2 mg/kg	Gupta and Kadel (1989a)
	Dermal	LD50	120 mg/kg	Ben-Dyke et al. (1970)
	Inhalation	LC50	85 mg/m^3	Tobin (1970)
Mice	Oral	LD50	2 mg/kg	Fahmy et al. (1970)
	SC	Lethal	2.5 mg/kg	Gupta (unpublished observations)
Rabbit	Dermal	LD50	885 mg/kg	Spencer (1973)
Guinea pig	Inhalation	LC50	43 mg/m^3/4 h	Anonymous (1967)
Dog	Oral	LD50	19 mg/kg	Tobin (1970)
	Inhalation	LC50	52 mg/m^3	Tobin (1970)
Sheep	Oral	Lethal	9 mg/kg	Osweiler et al. (1985)
Cattle	Oral	Lethal	18 mg/kg	Osweiler et al. (1985)
Chicken	Oral	LD50	6.3 mg/kg	Sherman et al. (1967)
Duck	Oral	LD50	415 μg/kg	Hudson et al. (1972)
Pheasant	Oral	LD50	4.2 mg/kg	Osweiler et al. (1985)
Quail	Oral	LD50	5 mg/kg	Osweiler et al. (1985)
Wild birds	Oral	LD50	420 μg/kg	Schafer et al. (1973)
	Dermal	LD50	100 mg/kg	Schafer et al. (1973)

manifestations of central and peripheral nervous systems origin by overstimulating muscarinic and nicotinic receptors (Cambon et al., 1979; Gupta & Kadel, 1989a, 1989b). In humans, symptoms of carbofuran toxicosis include salivation, diaphoresis, abdominal pain, chest tightness, drowsiness, dizziness, vomiting, muscular twitching, convulsions, and coma (Tobin, 1970; FMC, 1977). Similar clinical signs are observed in other species due to acute carbofuran poisoning.

PHARMACOKINETIC DATA

A detailed account of pharmacokinetic data on carbofuran is available in two recently published reviews (Ferguson et al., 1982; Anonymous, 1988). More detailed literature is available about the absorption (Ahdaya et al., 1981; Shah et al., 1981, 1987a, 1987b; Ahdaya & Guthrie, 1982), distribution (Ahdaya et al., 1981; Shah et al., 1981; Ahdaya & Guthrie, 1982), metabolism (Dorough, 1968; Ivie & Dorough, 1968; Metcalf et al., 1968; Knaak et al., 1970; Ferguson et al., 1984), and excretion (Dorough, 1968; Marshall & Dorough, 1979; Shah et al., 1981; Ahdaya et al., 1981; Khan et al., 1987) of carbofuran. Hussain et al. (1990) showed excretion of carbofuran in the urine of grain farmers after dermal and inhalation exposure to carbofuran at low dose levels for 4 d. Farmers did not exhibit any toxic manifestations, though blood cholinesterase was significantly inhibited. In this

review, I describe the metabolism of carbofuran in detail, since the metabolites have serious impact on overall toxicity of carbofuran. Recent experimental studies provided convincing evidence that residue of carbofuran or its metabolites can also be bioavailable in sufficient amount to cause adverse biological effects in rats and mice, following the consumption of vegetables and beans having a bound residue of carbofuran (Khan et al., 1987; Mostafa et al., 1992).

METABOLISM OF CARBOFURAN

The metabolism of carbofuran has been studied in rats (Dorough, 1968; Lucier et al., 1972; Marshall & Dorough, 1977, 1979; Ferguson et al., 1984; Bartow et al., 1994), mice (Metcalf et al., 1968), cows (Ivie & Dorough, 1968; Knaak et al., 1970), hens (Hicks et al., 1970), fish (Bruce, 1972), worms (Gilman & Vardanis, 1974), and houseflies (Metcalf et al., 1968; Dorough, 1968). A schematic representation of metabolic pathways of carbofuran in mammals, insects, and plants is shown in Figure 2.

Metabolism in Experimental Animals

In general, metabolism of carbofuran appears to involve hydroxylation and/or oxidation reactions that result in the formation of carbofuran phenol, 3-hydroxycarbofuran, 3-hydroxycarbofuran-7-phenol, 3-ketofuran, and/or 3-ketofuran-7-phenol (Dorough, 1968; Metcalf et al., 1968).

The key metabolites are produced by hydroxylation at the benzylic carbon to give 3-hydroxycarbofuran, which is oxidized to the 3-ketocarbofuran when not blocked by formation of conjugates. Dorough (1968) and Metcalf et al. (1968) identified the metabolites with certainty: 3-hydroxycarbofuran, 3-ketocarbofuran, and their respective 7-hydroxy hydrolysis products. These phenols, together with carbofuran phenol, are present in the free state and have also been identified as conjugates, principally the glucosides, in various biological systems (Metcalf et al., 1968). Recent in vitro findings of Bartow et al. (1994) suggested that cytochrome P-450 2E1 is the major isoenzyme responsible for 3-hydroxycarbofuran formation. The isoenzyme activity was found to be much greater in the liver than in the lung. There is also evidence of the formation of trace amounts of N-CH_2OH derivatives in mammals and insects produced from carbofuran or its 3-hydroxy or 3-keto derivatives (Metcalf et al., 1968). Dorough (1968) and Metcalf et al. (1968) also demonstrated various carbofuran metabolites (3-hydroxycarbofuran, 3-ketocarbofuran, 3-ketocarbofuran phenol, carbofuran phenol, and conjugate of 3-ketocarbofuran) in the urine of mice and rats orally dosed with radiolabeled carbofuran.

In an extensive investigation, Marshall and Dorough (1979) revealed that oral exposure of rats to labeled [^{14}C]carbofuran resulted in enterohepatic circulation of some metabolites. Radiocarbon in the urine of carbofuran-treated rats consisted largely of sulfate and glucuronide conjugates of carbofuran

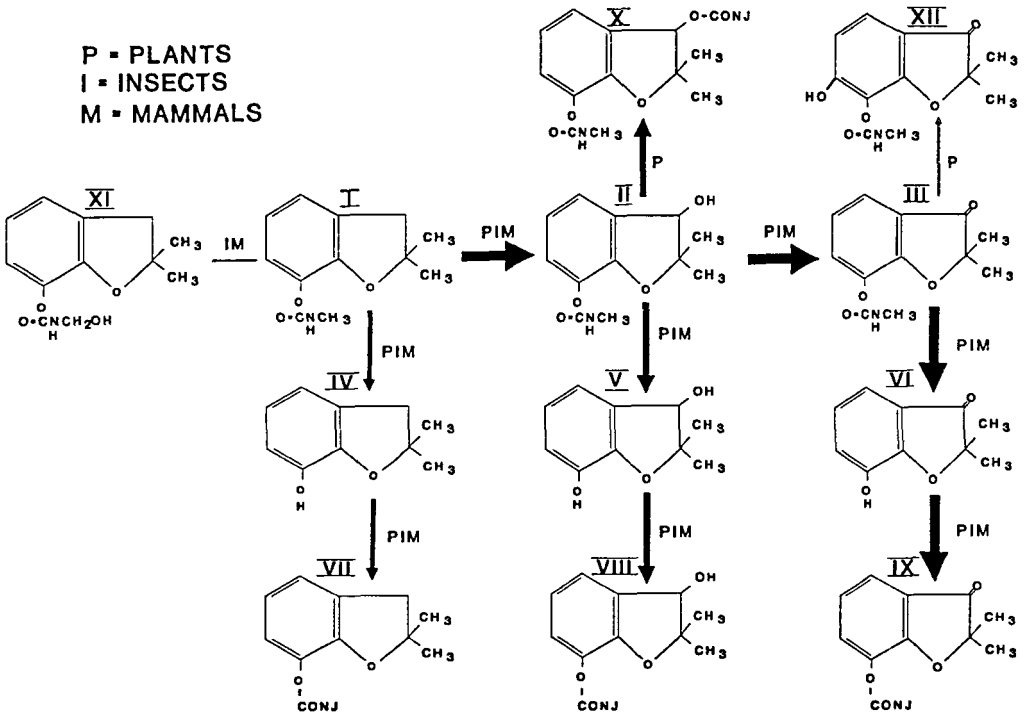


FIGURE 2. Metabolic pathways of carbofuran. I, 2,2-dimethyl-2,3-dihydrobenzofuranyl-7-*N*-methylcarbamate (carbofuran); II, 2,2-dimethyl-3-hydroxy-2,3-dihydrobenzofuranyl-7-*N*-methylcarbamate (3-hydroxycarbofuran); III, 2,2-dimethyl-3-keto-2,3-dihydrobenzofuranyl-7-*N*-methylcarbamate (3-ketocarbofuran); IV, 2,2-dimethyl,7-hydroxy-2,3-dihydrobenzofuran (carbofuran phenol); V, 2,2-dimethyl-3,7-dihydroxy-2,3-dihydrobenzofuran (3-hydroxycarbofuran phenol); VI, 2,2-dimethyl-7-hydroxy-3-keto-2,3-dihydrobenzofuran (3-ketocarbofuran phenol); VII, conjugate of carbofuran phenol (IV); VIII, conjugate of 3-hydroxycarbofuran phenol (V); IX, conjugate of 3-ketocarbofuran phenol (VI); X, conjugate of 3-hydroxycarbofuran (II); XI, 2,2-dimethyl-2,3-dihydrobenzofuranyl-7-*N*-hydroxymethylcarbamate (*N*-hydroxymethyl carbofuran); XII, 2,2-dimethyl-2,3-dihydro-3-keto-6-hydroxybenzofuranyl-7-*N*-methylcarbamate (3-keto-6-hydroxy carbofuran). "Conj" denotes glucuronide and sulfate conjugates. These metabolites are produced by oxidative, hydrolytic, and conjugative enzymatic reactions. M, in mammals; I, in insects; and P, in plants. Broad arrows represent major pathways. From Metcalf et al. (1968).

hydrolytic derivatives. However, the bile contained predominantly 3-hydroxycarbofuran glucuronide, a metabolite having the carbamate ester linkage intact, which may be cleaved to yield a potent anticholinesterase aglycone. Since enterohepatic cycling of glucuronides involves cleavage of the conjugate in the gut, biliary excretion may lead to increased systemic activity of toxic carbamate metabolites. 3-Hydroxycarbofuran is an important plasma and urine metabolite. The plasma elimination $t_{1/2}$ of the hydroxyderivative (64 min) is approximately twice as long as that of carbofuran (29 min). Thus, these findings suggest that enterohepatic circulation may be a key factor in maintaining anticholinesterase activity after the parent material no longer exists in the body (Marshall & Dorough, 1979; Ferguson et al., 1984).

Metabolism in Lactating Animals

In order to assess the potential risk to humans of carbofuran and its metabolites through contaminated milk, Ivie and Dorough (1968) and Knaak et al. (1970) studied the metabolism and elimination of labeled [^{14}C]carbofuran in lactating cows. Carbofuran was altered by oxidation of the number 3 carbon and of the *N*-methyl group, hydrolysis of the ester linkage, and conjugation of metabolites containing a hydroxyl group. Carbofuran metabolites in the milk were the 3-hydroxycarbofuran, 3-ketocarbofuran, and 3-hydroxy-*N*-hydroxymethyl derivatives of carbofuran, which were found in both the free and conjugated forms. 3-Hydroxycarbofuran, 3-ketocarbofuran phenol, and carbofuran phenol were the most prominent products, accounting for 70–80% of the radioactive content of the individual milk samples. Conjugated 2,3-dihydro-2,2-dimethyl-3-keto-7-hydroxybenzofuran was the major hydrolytic product of carbofuran in the milk. The same metabolites were also found in the urine and feces. 3-Hydroxycarbofuran was one of the most rapidly formed metabolites and 3-ketocarbofuran phenol was one of the end products. It was noted that of a single oral dose of carbofuran fed to a cow, approximately 0.2% is eliminated in the milk, 0.7% in the feces, and 94% in the urine.

Studies conducted on lactating goats by Tejada et al. (1988) revealed that the radioactive carbofuran residue was secreted in the milk at very low levels. Tissues of these goats also contained very low levels of residue. Only about 1% of the dose was eliminated in the feces, whereas the majority of the dose was rapidly excreted in the urine. It was concluded that proper usage of carbofuran in crops consumed by goats will present a minimum risk to goats and to humans who consume meat or milk from exposed goats.

Metabolism in the Environment (Soil and Water)

In soils, carbofuran is converted more by chemical than by microbial action. While carbofuran is one of the most rapidly degraded insecticides, its spontaneous degradation product, 3-hydroxycarbofuran, is moderately persistent. 3-Hydroxycarbofuran then breaks down into 3-ketocarbofuran, which is very short-lived (Miles et al., 1981).

Greenhalgh and Balanger (1981) studied the metabolism, uptake, and residue persistence of carbofuran in soils. The half-life of carbofuran was reported to be 15–38 d. Both 3-hydroxycarbofuran and 3-ketocarbofuran were detected as transformation products, the former reaching maximum levels between 1 and 7 d and the latter between d 16 and 36. The $t_{1/2}$ of carbofuran in sediment is between 1 and 2 mo (National Research Council of Canada, 1979). In a field experiment conducted at Beltsville, MD, during 1986–1988, Isensee et al. (1990) demonstrated that carbofuran residue persisted only for a short period (3 mo) after its application. The residues in unconfined (<1.5 m deep) groundwater were about 2 to 4 times higher than in confined (<3 m deep) groundwater. In similar experiments conducted in

California, no carbofuran was detected at any level in the soil strata to a depth exceeding 60 ft (Zalkin et al., 1984). Carbofuran was reported to move rapidly through the first meter of soil, and its short persistence prevented accumulation in groundwater.

The critical factors involved in the degradation of carbofuran in soils include physicochemical properties, soil type, soil temperature, moisture content, depth of groundwater, pH, and rainfall timing relative to carbofuran application. Several studies revealed that degradation of carbofuran was greater in soils with higher organic carbon content (Sukop & Cogger, 1992), alkaline pH, and hot, humid, rainy, and flooded conditions (Talekar et al., 1977; Venkateswarlu et al., 1977). On the other hand, persistence of carbofuran was increased in soil by certain factors including soil incorporation, use of granular formulations, low soil pH, and low soil temperature and moisture (CDPR, 1990, 1991). The effects of physicochemical properties of clay-loam and sandy-loam soils on the LC50 values of carbofuran were tested against *Folsomia candida*. The LC50 value was found to be 1.96 ppm in clay-loam soil compared with 0.69 ppm in sandy-loam soil (Achik et al., 1989). The authors concluded that under field conditions, the climate and the soil type affected the persistence and toxicity of carbofuran.

It was interesting to note that after 30 d of carbofuran application, carbofuran levels in soil previously treated with this insecticide were less than in those treated for the first time (Greenhalgh & Balanger, 1981). This was due to different induction times for the growth of carbofuran-degrading microorganisms in the soils. In similar experiments, Dzantor and Felsot (1989) demonstrated that pretreatment of a Drummer–Catlin soil mixture with carbofuran or other structurally similar carbamates enhanced biodegradation of their subsequent treatment. Several microbial biomass assays showed an increase in specific carbofuran-degrading bacteria in soils that were pretreated with carbofuran. Racke and Coats (1988) investigated the comparative degradation of five carbamate insecticides in soil as affected by enhanced microbial degradation. Soils with prior field exposure to carbofuran, cloethocarb, or several carbamates contained adapted microbial populations capable of rapidly degrading carbofuran. The persistence of aldicarb and its oxidative metabolites aldicarb sulfoxide and aldicarb sulfone was not dramatically altered in soils with enhanced carbofuran degradation. The authors suggested that although cross-adaptation for enhanced degradation exists within the carbamate insecticide class, structural similarity may play a role in modifying the expression of enhanced degradation in soil.

The metabolism of carbofuran in water can be expected to be similar to that in soil, though not much work has been done on it. In water, hydrolysis is the primary breakdown mechanism for carbofuran. Carbofuran is stable in water at low and neutral pH, but the rate of hydrolysis increases rapidly with increasing pH. Carbofuran has a hydrolysis $t_{1/2}$ of 35 d at pH 7.0 and 350 d at pH 6.0. Carbofuran is also degraded through photolysis and is not likely

to accumulate in water exposed to sunlight. Carbofuran does not bioaccumulate (National Research Council of Canada, 1979).

No studies were located regarding metabolism of carbofuran in the air.

Mechanism of Toxicity

Carbofuran elicits acute intoxication by virtue of reversible inhibition (carbamylation) of acetylcholinesterase (AChE) (Casida, 1963; O'Brien, 1967; Yu et al., 1972; Kuhr & Dorough, 1976; Gupta & Kadel, 1989a, 1989b), which hydrolyzes acetylcholine (ACh), a neurohumoral transmitter. The inhibition of AChE consequently leads to excessive ACh accumulation at the synapses and neuromuscular junctions, resulting in overstimulation of ACh receptors (muscarinic and nicotinic), which could ultimately end in death due to respiratory failure.

The mechanism by which carbofuran, like other carbamates, inhibits AChE is shown in Figure 3 and is similar to that of the substrate reaction (Wilson et al., 1960); the equation can be written as follows:

$$EH + CX \underset{k_{-1}}{\overset{k_{+1}}{\rightleftarrows}} EHCX \xrightarrow{k_2} EC + HX \xrightarrow[H_2O]{k_3} EH + COH$$

$$\underbrace{\qquad\qquad\qquad\qquad}_{k_i}$$

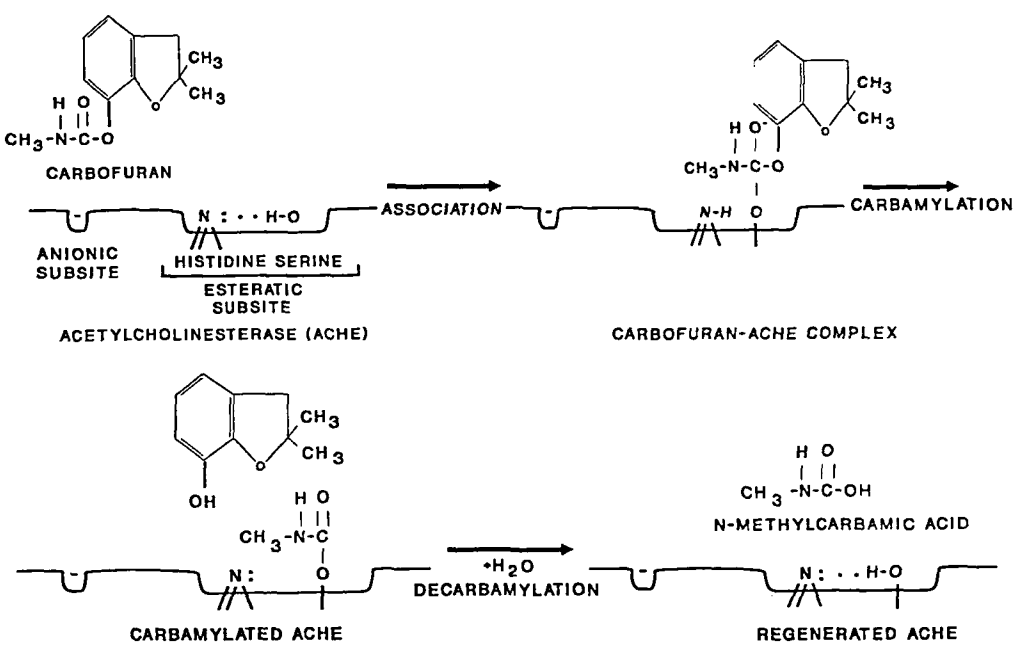


FIGURE 3. Steps involved in the reversible inhibition of AChE (carbamylation) by carbofuran and reactivation of AChE (decarbamylation).

where EH is the AChE enzyme, CX is the carbofuran with carbamylating radical C, and COH the carbamic acid; k_{+1} and k_{-1} are the binding constants; k_2 the carbamylation rate constant (Hastings, 1970); k_i the bimolecular rate constant governing the overall rate of inhibition; and k_3 the decarbamylation constant, which controls the enzyme recovery. The rate of carbamylation is more important than binding to the enzyme.

When carbofuran serves as alternate substrate, the alcohol moiety is cleaved, giving rise to the carbamylated AChE. In contrast to the acetylated AChE, carbamylated AChE is more stable (Wilson & Harrison, 1961). Sequestration of the AChE in carbamylated form thus precludes the hydrolysis of ACh, leading to ACh accumulation. Potent AChE inhibition results from rapid carbamylation (Yu et al., 1972; Kuhr & Dorough, 1976), as the carbamylation rate constant (k_i) is directly correlated with toxicity (Ferguson et al., 1984). The IC50 value for AChE inhibition by carbofuran was reported to be 3.3×10^{-8} (Dorough, 1968). IC50 values for AChE inhibition by carbofuran and other carbamates and organophosphates are summarized in Table 3. In general, IC50 values for carbamates decrease as the side chain becomes longer and bulkier (Soreq & Zakut, 1989). It should be noted that variation in IC50 values for different inhibitors could be due in part to different tissues and experimental conditions used, such as incubation time, temperature, pH, etc.

The time course of AChE inhibition in discrete brain regions and diaphragm muscle of rats acutely intoxicated with carbofuran (1.5 mg/kg sc) is shown in Figure 4. Among brain regions, cortex AChE was maximally inhibited, whereas striatum AChE was least affected. In earlier studies, carbofuran-induced AChE inhibition was demonstrated by several investigators in male rats (Ferguson et al., 1984), pregnant rats and conceptuses (Cambon et al., 1979, 1980), mice (Gupta et al., 1984), cattle and sheep (Palmer & Schlinke, 1973), and humans (Tobin, 1970). Both in vitro and in vivo studies provided convincing evidence that in addition to carbofuran, its major oxidative metabolite, 3-hydroxycarbofuran, strongly inhibits AChE activity (Dorough, 1968; Marshall & Dorough, 1979; Ferguson et al., 1984). 3-Hydroxycarbofuran possesses in vitro anticholinesterase activity within one order of magnitude of the parent compound (Metcalf et al., 1968). Marshall and Dorough (1979) and Ferguson et al. (1984) found the toxicity of this metabolite to be similar to that of carbofuran, since the two compounds have similar LD50 values (McCarthy, 1975).

Recovery of AChE from carbofuran-induced inhibition, that is, decarbamylation, is quite rapid, since recovery simply requires dissociation of the methylcarbamyl moiety from the enzyme (O'Brien et al., 1966; O'Brien, 1969). A short enzyme recovery period was explained in part by kinetics of the reversible AChE–carbofuran complex. It has been reported that the in vivo carbofuran hydrolysis rate constant ($K_{m1} = 0.028$ min^{-1}, $t_{1/2} = 25$ min), representing all esterases active in carbofuran hydrolysis (Ferguson et al., 1984), is similar to that of the in vitro ($t_{1/2} = 20$–63 min) value, suggesting

TABLE 3. Comparative IC50 for Acetylcholinesterase (AChE) or Cholinesterase (ChE) Inhibition by Carbamates and Organophosphates

Compound	Species	Tissue	IC50[a] (*M*)	Reference
		Carbamates		
Carbofuran	Rat	Blood	3.3×10^{-8}	Dorough (1968)
	Rat	Blood	1.2×10^{-8}	Ferguson et al. (1984)
	Human	Blood	4.6×10^{-6}	Soreq and Zakut (1989)
	Fly	Head	2.5×10^{-7}	Metcalf et al. (1968)
Bendiocarb	Human	Blood	1.45×10^{-5}	Soreq and Zakut (1989)
Benfuracarb	Human	Blood	1.17×10^{-4}	Soreq and Zakut (1989)
Carbaryl	Human	Plasma	1.2×10^{-5}	Villeneuve (1971)
Carbosulfan	Human	Blood	3.07×10^{-4}	Soreq and Zakut (1989)
DMC[b]	Rat	Brain	1.62×10^{-9}	Patocka and Bajgar (1987)
Furathiocarb	Human	Blood	7.25×10^{-6}	Soreq and Zakut (1989)
Neostigmine methylsulfate (Prostigmine)	Human	Blood	1.7×10^{-7}	Soreq and Zakut (1989)
Physostigmine sulfate (Eserine)	Human	Blood	2.2×10^{-7}	Soreq and Zakut (1989)
	Human	RBC	2.45×10^{-8}	Thomsen et al. (1991)
Physostigmine salicylate	Eel	—	4.0×10^{-9}	Brossi et al. (1986)
Pyridostigmine bromide	Human	Blood	6.8×10^{-9}	Soreq and Zakut (1989)
	Human	RBC	3.16×10^{-7}	Thomsen et al. (1991)
		Organophosphates		
BW284C51	Human	RBC	6.61×10^{-8}	Thomsen et al. (1991)
Chlorpyriphos oxon	Bovine	RBC	4.6×10^{-9}	Sultatos et al. (1982)
	Mice	Brain	3.6×10^{-9}	Sultatos et al. (1982)
DFP	Human	Plasma	5.01×10^{-8}	Villeneuve (1971)
Methyl chlorpyriphos oxon	Bovine	RBC	2.2×10^{-6}	Sultatos et al. (1982)
	Mice	Brain	1.73×10^{-6}	Sultatos et al. (1982)
Guthoxon	Human	Plasma	6.31×10^{-5}	Villeneuve (1971)
Hinosan	Bovine	RBC	1.17×10^{-4}	Malik et al. (1978)
Phosphamidon	Human	Plasma	6.7×10^{-7}	Villeneuve (1971)
iso-OMPA	Human	RBC	0.85×10^{-3}	Thomsen et al. (1991)
Malathion	Bovine	RBC	3.87×10^{-5}	Malik et al. (1978)
Malaoxon	Bovine	RBC	5.86×10^{-8}	Cohen et al. (1985)
	Rat	RBC	2.71×10^{-8}	Cohen et al. (1985)
	Bovine	Brain	6.36×10^{-8}	Cohen et al. (1985)
	Rat	Brain	3.65×10^{-8}	Cohen et al. (1985)
Parathion	Human	Plasma	2.43×10^{-5}	Villeneuve (1971)
Paraoxon	Bovine	RBC	1.44×10^{-8}	Cohen et al. (1985)
	Human	Plasma	1.88×10^{-8}	Villeneuve (1971)
	Rat	RBC	0.32×10^{-8}	Cohen et al. (1985)
	Bovine	Brain	1.66×10^{-8}	Cohen et al. (1985)
	Rat	Brain	0.53×10^{-8}	Cohen et al. (1985)
	Rat	Brain	2.6×10^{-8}	Kemp and Wallace (1990)
	Rat	Brain	1.24×10^{-8}	Milatovic and Dettbarn (1994)
	Rat	Diaphragm	2.27×10^{-8}	Milatovic and Dettbarn (1994)
	Hen	Brain	2.8×10^{-8}	Kemp and Wallace (1990)
Sumithion	Bovine	RBC	4.1×10^{-5}	Malik et al. (1978)

[a]Molar concentration causing 50% AChE or ChE inhibition (IC50).
[b]3-Diethylaminophenyl-*N*-methylcarbamate (DMC).

rapid enzyme recovery (O'Brien et al., 1966; O'Brien, 1969; Aldridge, 1971). Enzyme recovery is faster in skeletal muscle than in brain regions (Figure 4).

BIOCHEMICAL ALTERATIONS

Enzymatic

Carbofuran, in addition to causing inhibition of target enzyme AChE (Figures 3 and 4), inhibits the activities of other nonspecific serine-containing enzymes, such as carboxylesterases (CarbEs) and butyrylcholinesterases (BuChE). This suggested tremendous nonspecific binding of carbofuran (Gupta & Kadel, 1989a, 1989b). Inhibition of CarbE was demonstrated in both neuronal (brain) and nonneuronal (skeletal muscle, liver, and plasma) tissues (Figure 5). Carbofuran caused significantly greater inhibition of this enzyme in plasma and peripheral tissues than in brain. This indicates a nonspecific binding in peripheral tissues as a protective mechanism by leaving very little free concentration of carbofuran for critical AChE inhibition in the brain.

Our recent findings showed that acute carbofuran intoxication in rats (1.5 mg/kg sc) caused significant perturbations in mitochondrial/cytoplasmic enzymes (creatine kinase, CK; lactic dehydrogenase, LDH) and their isoen-

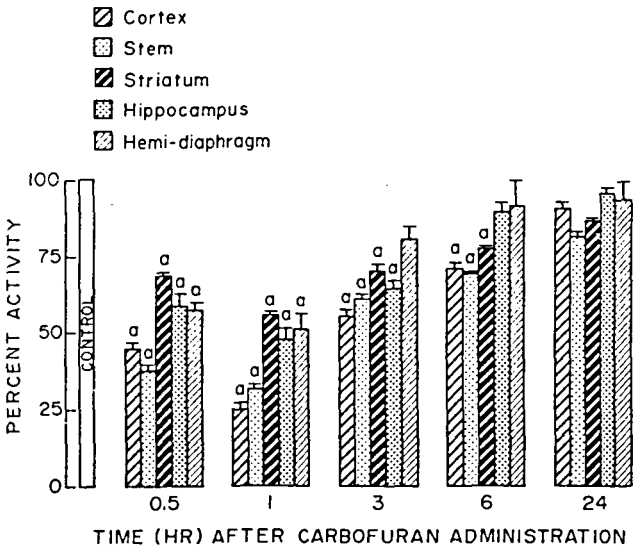


FIGURE 4. Time course of AChE (percent activity) in discrete brain regions and hemidiaphragm muscle of rats acutely intoxicated with carbofuran (1.5 mg/kg sc). a, Statistically significant difference between controls and carbofuran-treated rats ($p < .01$). From Gupta and Kadel (1989a).

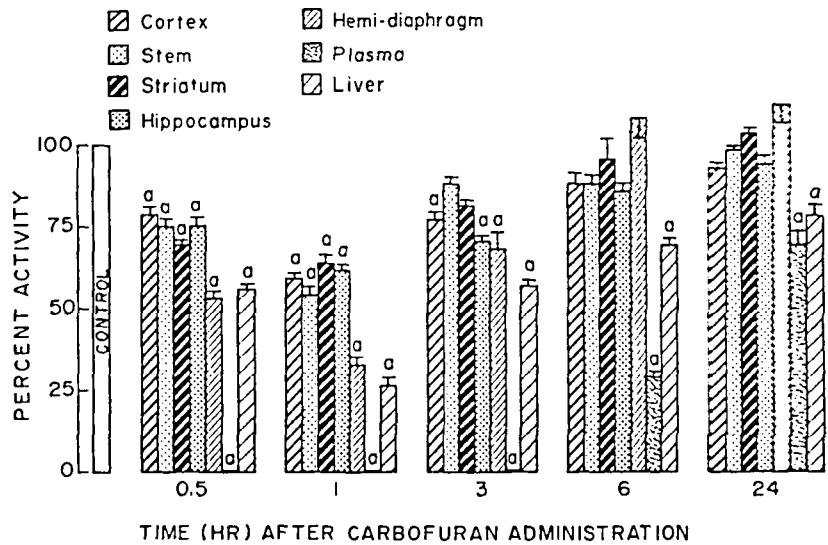


FIGURE 5. Time course of CarbE (percent activity) in discrete brain regions, hemidiaphragm, plasma, and liver of rats acutely intoxicated with carbofuran (1.5 mg/kg sc). a, Statistically significant difference between controls and carbofuran-treated rats ($p < .01$). From Gupta and Kadel (1989a).

zymes and subforms in various vital organs, which consequently showed changes in serum. Gupta et al. (1991a, 1994b) demonstrated that acute carbofuran intoxication in rats caused significant elevation of creatine kinase (CK, EC.2.7.3.2) activity, along with an increase in CK isoenzymes (CK-BB and CK-MM) activity in serum (Figure 6) as a result of their leakage from the brain, heart, and skeletal muscles [soleus, extensor digitorum longus (EDL), and diaphragm]. Normally, all three skeletal muscles have only one CK isoenzyme (CK-MM), which consists of only one CK-MM subform (CK-MM3). In contrast, serum has three distinct CK-MM subforms (CK-MM1, 6.3%; CK-MM2, 24%; and CK-MM3, 69.7%). Carbofuran intoxication caused leakage of CK-MM3 from muscles into serum, where it enhanced the sequential conversion of MM3 to MM2 and ultimately to MM1 by elevating carboxypeptidase-N activity (Gupta et al., 1994b).

In another similar investigation, Gupta et al. (1991b, 1994b) demonstrated that acute carbofuran intoxication (1.5 mg/kg body weight sc) caused a twofold increase in lactic dehydrogenase (LDH) activity, along with characteristic alterations in electrophoretically distinct LDH isoenzymes patterns in the serum of rats (Figure 7). Analyses of vital organs (brain, heart, liver, kidney, and skeletal muscles) revealed characteristic changes in LDH isoenzymes patterns, indicating tissue-specific damages, which were consequently reflected in serum. For the details of time courses of CK, LDH, and their isoenzymes, see Gupta et al. (1991a, 1991b, 1994a, 1994b). Leakage of CK, LDH, and their isoenzymes from tissues into serum is due to depletion

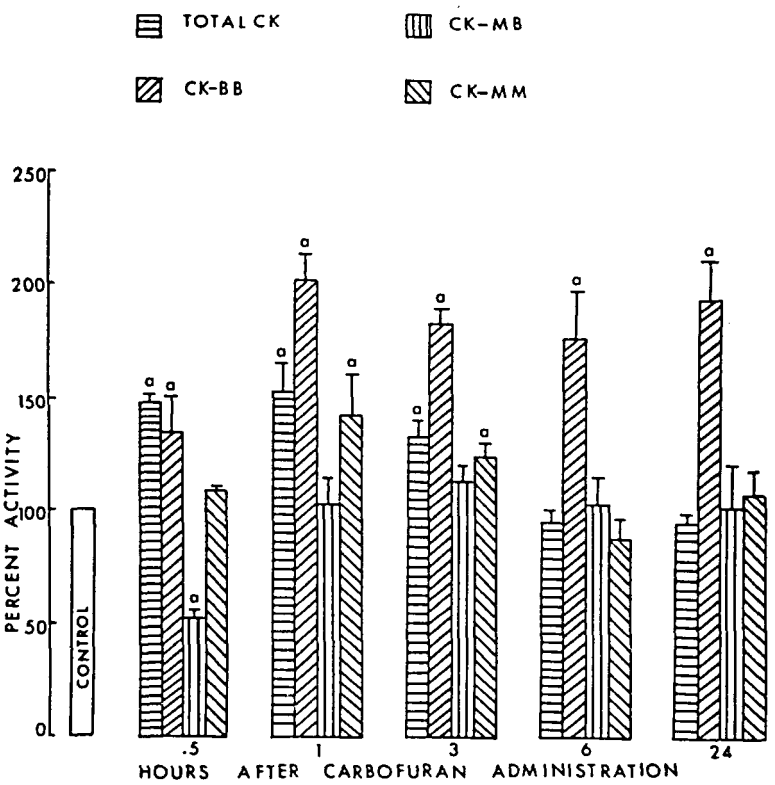


FIGURE 6. Time course of creatine kinase (CK) and CK isoenzymes in serum of rats acutely intoxicated with carbofuran (1.5 mg/kg sc). a, Statistically significant difference between controls and carbofuran-treated rats ($p < .01$). From Gupta et al. (1991a).

of ATP and PCr (Table 4), which are required to maintain cell membrane permeability and integrity (Woodman, 1981; Goad et al., 1994; Gupta et al., 1991a, 1994a, 1994b; Andreoli, 1993).

Nonenzymatic

Neurotransmitters In an experimental study conducted in mice, Gupta et al. (1984) examined the effects of multiple ip doses of carbofuran (0.25 mg/kg) on the concentrations of ACh, gamma-aminobutyric acid (GABA), epinephrine, norepinephrine, dopamine, and 5-hydroxytryptamine (5-HT) in the brain. Carbofuran treatment caused significant increases in these neurotransmitters, suggesting that the observed neurochemical perturbations in the brain might be associated with central nervous system (CNS) depressant action induced by carbofuran.

Proteins, Lipids, and Lipoproteins Gupta et al. (1986) investigated the effects of carbofuran on the concentrations of total lipids and its different fractions and on lipase activity in mice treated with multiple ip doses (0.125,

0.25, or 0.5 mg/kg) for 2, 4, and 6 wk. Treated mice showed elevated levels of total lipids, cholesterol (total and free), phospholipids (total and fractions—lecithin, lysolecithin, phosphatidyl ethanolamine, and lysophosphatidyl-ethanolamine), and triglyceride in liver, kidney, and serum. Total phospholipid and its fractions decreased significantly in brain. Lipase activity decreased significantly in liver and serum. These authors concluded that such disorders of lipid levels, especially in the brain, might be associated with CNS depressant action, and structural and functional toxicity of other tissues by carbofuran.

Recently we examined the acute effects of carbofuran (1.5 mg/kg sc) on proteins, lipids, and lipoproteins in liver and serum of rats (Gupta et al., 1994c). Analyses of globulin fractions revealed remarkable changes. In liver, the levels of alpha-2, alpha-3, and gamma fractions were elevated while alpha-1 was reduced, whereas in serum, alpha-1 and alpha-3 fractions were elevated. A transient increase in total protein and albumin was noted only in liver. Carbofuran produced significant increases in triglycerides and cholesterol in liver, which were also seen in serum. In both the liver and serum, the levels of low-density lipoprotein cholesterol were reduced while the values of very-low-density lipoprotein cholesterol were elevated. The concentrations of high-density lipoprotein cholesterol were drastically reduced in liver

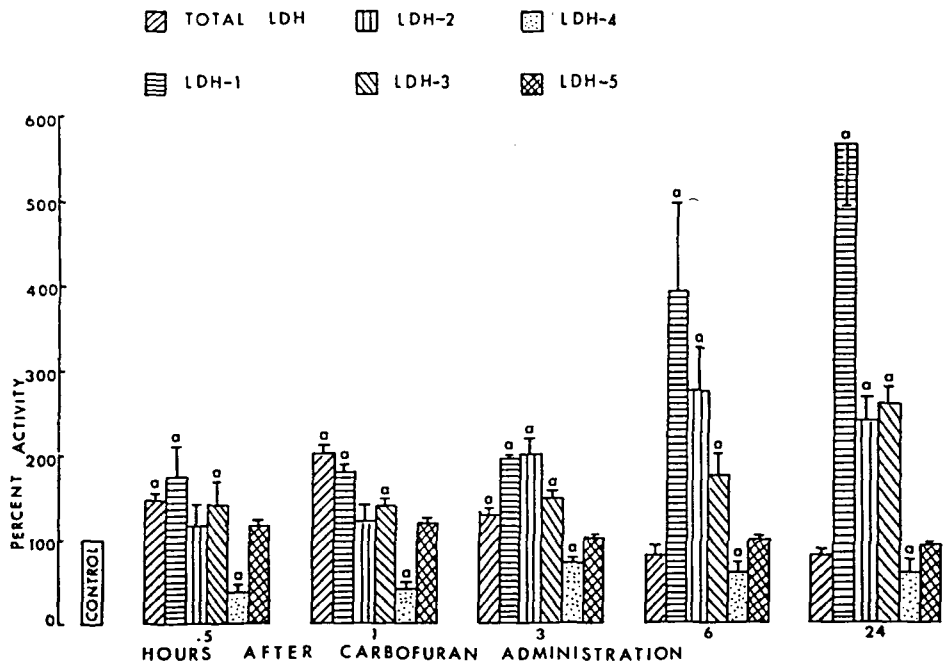


FIGURE 7. Time course of lactic dehydrogenase (LDH) and LDH isoenzymes in serum of rats acutely intoxicated with carbofuran (1.5 mg/kg sc). a, Statistically significant difference between controls and carbofuran-treated rats ($p < .01$). From Gupta et al. (1991b).

TABLE 4. Effects on Energy-Related Parameters in the Hemidiaphragm of Rat at 1 h after Carbofuran (1.5 mg/kg sc) Administration

Parameters	Control	Carbofuran
ATP (μmol/g)	5.66 ± 0.09 (100)	4. 06 ± 0.11[a] (72)
ADP (μmol/g)	0.64 ± 0.02 (100)	0.62 ± 0.04 (97)
AMP (μmol/g)	0.21 ± 0.01 (100)	0.24 ± 0.02 (114)
TAN (μmol/g)	6.51 ± 0.12 (100)	5.18 ± 0.29[a] (80)
ATP/ADP ratio	8.93 ± 0.20 (100)	6.61 ± 0.45[a] (74)
ATP/AMP ratio	27.53 ± 1.95 (100)	16.92 ± 0.98[a] (61)
Energy charge	0.92 ± <0.01 (100)	0.88 ± <0.01 (96)
PCr	8.60 ± 0.45 (100)	6.08 ± 0.19[a] (71)
Cr	16.11 ± 0.70 (100)	14.30 ± 0.60 (89)
TCC	24.70 ± 0.67 (100)	20.39 ± 0.41[a] (82)
PCr/Cr	0.54 ± 0.03 (100)	0.43 ± 0.03[a] (80)

Note. Values are means ± SEM (n = 4–5). Abbreviations: TAN, total adenine nucleotides (ATP + ADP + AMP); energy charge, ATP + 1/2 ADP/(ATP + ADP + AMP); PCr, phosphocreatine; Cr = creatine; TCC, total creatine compounds (PCr + Cr).

[a]Statistically significant difference between controls and carbofuran-treated rats ($p < .01$).

(23% of control) with a proportional rise in serum (176%). Leakage of hepatic constituents into serum was due to depletion of ATP (62%) and PCr (78%), which are required to retain intracellular constituents by maintaining cell membrane permeability, as discussed earlier.

High-Energy Phosphates Skeletal muscles, brain, and heart are the main targets for the toxicity of carbofuran and other anticholinesterase pesticides (Petras, 1981; Lemercier et al., 1983; Gupta & Kadel, 1989a, 1989b). These organs are also known to consume greater energy than any other organs in the body. In fact, muscle fasciculations are the most profound feature of carbofuran's toxicity (Gupta & Kadel, 1989a, 1989b). Following

intense muscle contractions, muscle metabolism undergoes tremendous changes. Carbofuran acute poisoning (1.5 mg/kg sc) in male rats caused marked depletion of ATP and PCr in skeletal muscles (soleus, EDL, and diaphragm) (Gupta et al., 1991a, 1994b). Significant declines in the levels of total adenine nucleotides (ATP + ADP + AMP) and total creatine compounds (PCr + Cr) were also evident. Decreases in the corresponding ratios of ATP/ADP, ATP/AMP, and PCr/Cr were therefore suggestive of greater utilization of ATP and PCr in response to their increased demand for maintaining high-frequency muscle fasciculations. Decrease in PCr was much greater than in ATP in all muscles, since the synthesis of PCr is only from ATP (CK: forward Lohmann reaction); however, ATP can be generated not only from PCr (CK: reverse Lohmann reaction), but also from glycolysis, oxidative phosphorylation, or two ADP molecules (Hinkle & McCarty, 1978; Moraes & Meis, 1987). The energy charge, ATP + ½ADP/(ATP + ADP + AMP), an index of high-energy phosphate adequacy in muscles, remained unchanged. Some of the changes related to energy metabolism are summarized in Table 4. The alterations observed in muscles are also seen in liver, which is suggestive of involvement of the "cori cycle."

HEMATOLOGICAL CHANGES

In an extensive investigation, effects of carbofuran with multiple ip doses in mice (0.25 mg/kg) on a variety of hematological parameters [clotting time, hemoglobin (Hb) content, total red blood cells (RBC), total white blood cells (WBC), differential WBC count, platelets, erythrocyte sedimentation rate (ESR), hematocrit value, mean corpuscular volume (MCV), mean corpuscular Hb, and mean corpuscular Hb concentration] were investigated by Gupta et al. (1982). Carbofuran intoxication caused a significant decrease in Hb content, total RBC, platelets, ESR, and hematocrit value. The intoxication increased total WBCs and prolonged clotting time. Among WBCs, there was an increase in neutrophils and basophils and a decline in the lymphocytes. Bone marrow depression and splenic hyperplasia were also evident. Hussain et al. (1990) found no significant alterations in any hematological parameters (RBC, MCV, Hb content, hematocrit, WBC, lymphocytes, monocytes, and granulocytes) in farmers exposed with non-signs-producing doses of carbofuran for 4 d by dermal and inhalation routes.

IMMUNE RESPONSES

Changes in serum immunoglobulin (Ig) concentrations (indicators of immunocompetence) over the lifespan of mice exposed in utero to carbofuran (0.01 or 0.5 mg/kg in the diet daily throughout gestation) were studied by Barnett et al. (1980). All mothers gave birth to viable, overtly normal offspring at term. Determinations of five different classes of serum immunoglobulin concentrations (IgG1, IgG2a, IgG2b, IgA, and IgM) at 101, 400, and 800 d of age indicated transient, but consistent, disturbances of 2 Ig classes in offspring as a

result of carbofuran exposure. IgG1 concentrations of male offspring exposed to 0.5 mg/kg carbofuran were significantly elevated at 101 d but not at 400 or 800 d. IgG1 concentrations of female offspring exposed to 0.01 mg/kg carbofuran were significantly depressed at 101 d but not at 400 or 800 d. Changes in IgG2b levels were similar to those recorded for IgG1, but of smaller magnitude. Studies by Street and Sharma (1975) demonstrated alterations in cellular and humoral immune responses, that is, immunosuppression by carbofuran.

Flipo et al. (1992) evaluated immunotoxic potential in C57B1/6 inbred mice following acute exposure to carbofuran given singly or in combination with dieldrin and malathion. Their findings indicated the immunosuppressive potential of carbofuran or dieldrin and the immunopotentiating effect of malathion when exposed to a single insecticide. However, when mice were acutely exposed to a mixture of carbofuran and dieldrin, tests revealed a lack of any synergistic or additive effects on the immune response. These data suggested that the carbofuran/dieldrin mixture had an antagonistic effect on the humoral response to sheep red blood cells (SRBC) and macrophage phagocytic activity, in comparison with the action of each of the insecticides alone. No studies were located that investigated immunological effects in humans after exposure to carbofuran.

PLACENTAL TRANSFER AND FETAL AChE INHIBITION

In a series of experiments, Cambon and his colleagues (1979, 1980) demonstrated the transplacental transfer of carbofuran in mice and rats. Cambon et al. (1979) studied the acute effects of carbofuran (0.25 and 2.5 mg/kg by gastric intubation) in pregnant Sprague-Dawley rats on d 18 of gestation. The rats were sacrificed 1, 5, and 24 h after dosage. Signs of central and peripheral effects due to AChE inhibition occurred 5 min after carbofuran treatment. Carbofuran caused a significant decrease in the activity of AChE in most maternal and fetal tissues examined 1 h after treatment. Their findings revealed that AChE inhibition was more prominent in fetuses than in dams, due to accumulation of carbofuran in fetal tissues because of their inability to metabolize carbofuran. In another study, Cambon et al. (1980) evaluated the distribution of cerebral AChE isoenzymes of fetuses and pregnant rats in control and carbofuran-treated (on d 18 of gestation) groups. Their findings showed the existence of differences in cerebral AChEs between mother and fetus. Carbofuran caused a significant decrease of isoenzyme I in the mother, and a decrease of isoenzyme II in the fetus. The difference in sensitivity of cerebral AChE to carbofuran was thought to depend on differences in the fixation of carbofuran or its metabolite(s) on the fetal and maternal isoenzymes.

Recently, Klys et al. (1989) presented a case in which a pregnant woman recovered from carbofuran poisoning, following carbofuran ingestion, but the fetus died of severe pathological changes. Toxicological findings of the brain, liver, and kidney of the fetus revealed carbofuran in concentrations comparable with the mother's blood. These findings suggest easy permeation

of carbofuran through the placental barrier, and greater vulnerability of the fetus to carbofuran toxicity.

TERATOGENIC, REPRODUCTIVE, AND DEVELOPMENTAL EFFECTS

Pawar and Katdare (1984) evaluated the toxic and teratogenic effects of carbofuran on the embryos and tadpoles of frogs (*Microhyla ornata*) for a period of 96 h. Abnormalities observed in embryos and tadpoles were blisters on the body, distention of the body cavities, curvature of the body axis, poor blood circulation, poor pigmentation, retarded growth, loss of balance, and abnormal behavior. In general, tadpoles were more sensitive than the embryos. In an investigation where carbofuran was administered ig once daily to CD rats (0.05–5 mg/kg) on d 7–19 of gestation or to CD-1 mice (0.1–20 mg/kg) on d 6–16 of gestation, Courtney et al. (1985) revealed that at dose levels that were not maternally lethal, carbofuran did not produce fetotoxicity, fetolethality, or fetal malformations. Also, carbofuran was not found teratogenic in the CD rats or CD-1 mice at maternally nontoxic doses.

In a feed trial, carbofuran given to beagles for 1 yr at 0.25, 0.50, or 12.5 mg/kg/d caused aspermia in males at the 2 higher doses. Testicular degeneration in the males and uterine hyperplasia and hydrometria in females were observed with the highest doses of carbofuran (FMC, 1983). In a 3-generation study in which rats were fed carbofuran at 1 or 5 mg/kg/d, no adverse effects were observed on female or male fertility, gestation length, litter size or growth, or pup viability. However, with a higher dose, the survival of the first litter in all 3 generations was slightly lower by d 4 of lactation (FMC, 1980a). The no-observed-adverse-effect level (NOAEL) for reproductive effects was determined to be 1 mg/kg/d. In another study, no adverse effects were observed on the 28- or 800-d survival rates of mice whose mothers had been fed carbofuran at 0.01 or 0.5 mg/kg/d throughout gestation (Barnett et al., 1980).

Pregnant rats exposed to carbofuran at 1, 2.9, 5.8, 7.7, or 9.7 mg/kg/d (FMC, 1980b) or at 1, 3 or 8 mg/kg/d (FMC, 1981a) on d 6–19 of gestation did not show any observable clinical signs of toxicity or adverse effects on pup survival or visceral or skeletal development. Maternal body weight gains were reduced at the 2.9–9.7 mg/kg/d dosage levels in the first study and at the 3 and 8 mg/kg/d dosage levels in the second study. In similar experiments, rabbits exposed to carbofuran at 0.12, 0.5, or 2 mg/kg/d by gavage on d 6–18 of gestation showed no developmental effects in the offspring. No decreases in the numbers of fetuses or litters and no observable developmental or genetic abnormalities were noted. Dams at the highest dose showed a 20% reduction in weight gain (FMC, 1981b).

MUTAGENICITY AND CARCINOGENICITY

The data on mutagenicity of carbofuran are inconclusive. Mutagenicity testing of carbofuran by the Ames bacterial test indicated a negative result (U.S. EPA, 1985). However, in one study, in which carbofuran was applied

at up to 10 mg/plate with rat liver S9 activation, it was found mutagenic in *Salmonella typhimurium* strains TA98 and TA1538 (Moriya et al., 1983). Mutagenicity tests of carbofuran in other test systems were negative with one exception: Chinese hamster ovary (CHO) V79 cells. Wojciechowski et al. (1982) reported positive results at an unspecified dosage of carbofuran without, but not with, rat liver S9 activation.

Nelson et al. (1981) determined the mutagenicity of *N*-nitroso derivatives of carbofuran and its toxic metabolites (3-hydroxycarbofuran and 3-ketocarbofuran) by the Ames assay method with *S. typhimurium* strains TA98 and TA100. The nitroso derivatives of all three compounds responded similarly, giving a mutation ratio of 45 at 5 μg/plate on TA100. In addition, all three compounds produced chromosomal aberrations in CHO cells. Nitrosocarbofuran and 3-hydroxynitrosocarbofuran were also found capable of inducing large numbers of sister chromatid exchanges in the same cells.

Two-year dietary studies of carbofuran in rats at 0.5, 1, or 5 mg/kg/d (FMC, 1980c) and in mice at 3, 18.8, or 75 mg/kg/d (FMC, 1980d) revealed no evidence of carcinogenicity in either species. No studies were located regarding mutagenic or carcinogenic effects in humans after oral, dermal, or inhalation exposure to carbofuran.

INTERACTION WITH OTHER PESTICIDES

In agricultural practice, carbamate and organophosphate (OP) insecticides are often used in combination to control a wide range of insects, including the hard-to-kill resistant insects. Many times, in veterinary practice, these chemicals are also employed simultaneously as ectoparasiticides and as anthelmintics. Interaction of pesticides could take place during absorption, distribution, metabolism, and elimination. Unfortunately, the interaction of carbofuran has been studied with only a few pesticides.

In an in vitro interaction study, Iyaniwura (1990, 1991) reported three carbamate insecticides (carbofuran, oxamyl, and propoxur) as having an additive effect on AChE activity. In an extensive in vivo interaction study, Gupta and Kadel (1989a) investigated the interaction between carbofuran and *iso*-OMPA (an OP compound), considering AChE as a real target enzyme (active-site-directed inhibition) and carboxylesterase (CarbE) as a false target enzyme (nonspecific-site-directed inhibition). It was of interest to study the interaction with carbofuran, since it is not only an inhibitor of AChE and CarbE, but also a substrate for CarbE. Pretreatment of rats with *iso*-OMPA (1 mg/kg sc) 1 h prior to carbofuran (0.5 mg/kg sc) administration potentiated the carbofuran toxicity by more than threefold. Neither *iso*-OMPA nor carbofuran in the given doses produced any gross toxic signs. Rats receiving combined treatment, however, showed severe hypercholinergic signs with parasympathetic preponderance within 5–15 min following carbofuran administration. The severity was comparatively greater than that observed with an acute dose of carbofuran (1.5 mg/kg sc).

The observed potentiation of carbofuran toxicity was explained by more than one mechanism. One mechanism is that *iso*-OMPA occupied nonspecific binding sites (non-AChE-active serine sites such as CarbE) in the liver, plasma, muscle, brain, and elsewhere, which would normally be available for carbofuran. Irreversible occupation of these nonspecific sites by *iso*-OMPA would leave a high concentration of free carbofuran, resulting in a critical AChE inhibition (Figure 8). It also appears that plasma,

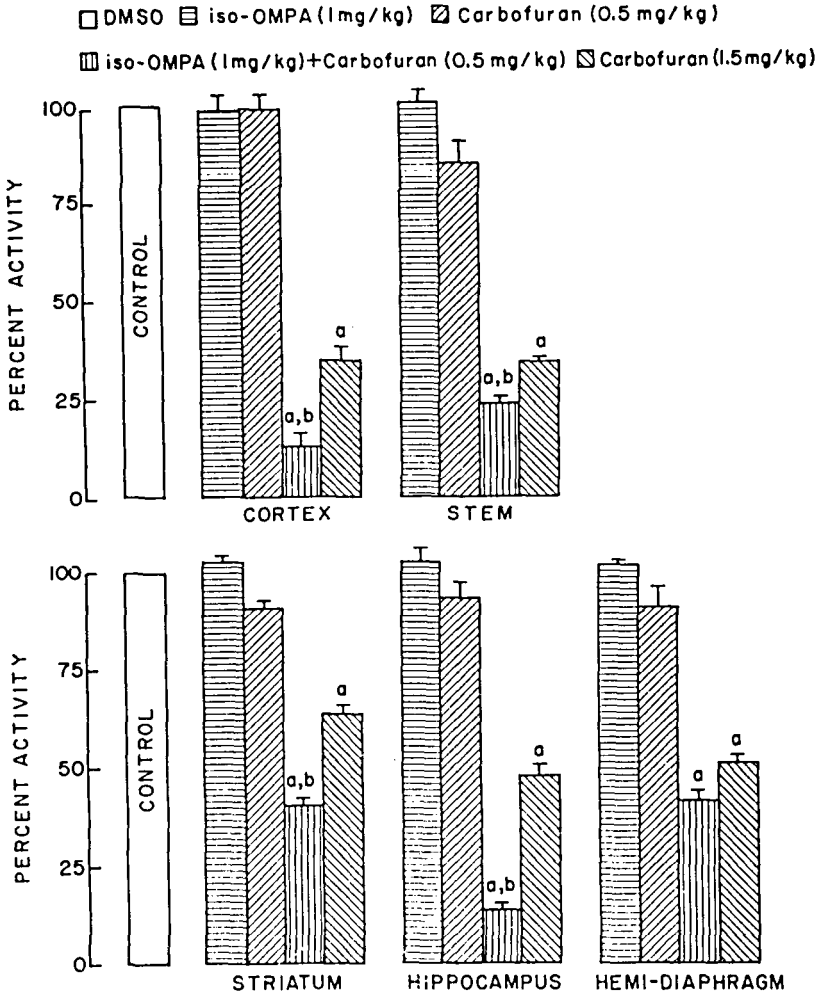


FIGURE 8. Effects of *iso*-OMPA and carbofuran administered singly or in combination on AChE (percent activity) in brain regions and hemidiaphragm. a, Statistically significant difference between controls and treated rats ($p < .01$). b, Statistically significant difference between rats treated with *iso*-OMPA (1 mg/kg sc) plus carbofuran (0.5 mg/kg sc) and carbofuran (1.5 mg/kg sc) ($p < .01$). From Gupta and Kadel (1989a).

liver, muscle, and brain are important sites for the detoxification of carbofuran.

Another important mechanism in the potentiation of carbofuran toxicity by *iso*-OMPA could be interference in the degradative metabolism of carbofuran by inhibiting carbamatase, or by the blocking of nonspecific binding to nonenzymatic proteins, which may result in raising the free carbofuran concentrations. Thus, simultaneous exposure to more than one insecticide may pose a serious threat to human as well as animal health, probably as a result of toxic interaction.

ANTIDOTAL THERAPY

Complete success in developing antidotal treatment against anticholinesterase poisoning has never been achieved, since there is no single therapeutic agent yet available that can antagonize the toxic effects, central as well as peripheral, evoked by overstimulation of both muscarinic and nicotinic ACh receptors. Treatment of carbofuran acute toxicosis, as with other carbamates, so far rests with only atropine sulfate, which readily antagonizes the muscarinic receptor-associated effects, such as salivation, lacrimation, urination, diarrhea, and, more importantly, tracheobronchial secretions. Atropine sulfate, however, does not antagonize the nicotinic-receptor-associated effects, such as muscle tremors, fasciculations, and convulsions. Further, atropine sulfate alone as a therapeutic agent is of very little or no value when poisoning involves a lethal dosage of carbofuran (Gupta & Kadel, 1989b). Nucleophilic agents, such as hydroxylamine, hydroxamic acid, and oximes, reactivate the inhibited AChE against OPs (Berry et al., 1959; Hobbiger & Sadler, 1959; Hobbiger et al., 1960), but are not effective against carbamates (Sanderson, 1961; Natoff & Reiff, 1973). In fact, pyridine-2-aldoxime methyl chloride (2-PAM) is contraindicated as an antidote for certain carbamates (Gupta, unpublished observations), as 2-PAM exacerbated the toxicity. Recently, we also found that diazepam accentuates carbofuran's toxicity. Therefore, until recently no antidotal treatment, other than atropine, was recommended against carbofuran or any other carbamates.

In a recent investigation, we found that a combined antidotal treatment consisting of memantine HCl (18 mg/kg sc) and atropine sulfate (16 mg/kg sc) provides complete protection and antagonism against acute carbofuran poisoning in rats (Gupta & Kadel, 1989b). Memantine protects from and antagonizes carbofuran poisoning in animals by multiple mechanisms: (1) protecting AChE activity or rapid reactivation from inhibition (Figure 9), (2) protecting CarbE activity, leaving free carbofuran for rapid elimination (Gupta & Kadel, 1989b), (3) direct effects to prevent neural hyperexcitability (McLean et al., 1992), and (4) reversible blockage of neuromuscular transmission (Masuo et al., 1986). In addition, combined treatment of memantine and atropine provides protection and reversal of alterations in biomarker enzymes/isoenzymes (CK, LDH, and transaminases) by maintaining cell membrane characteristics,

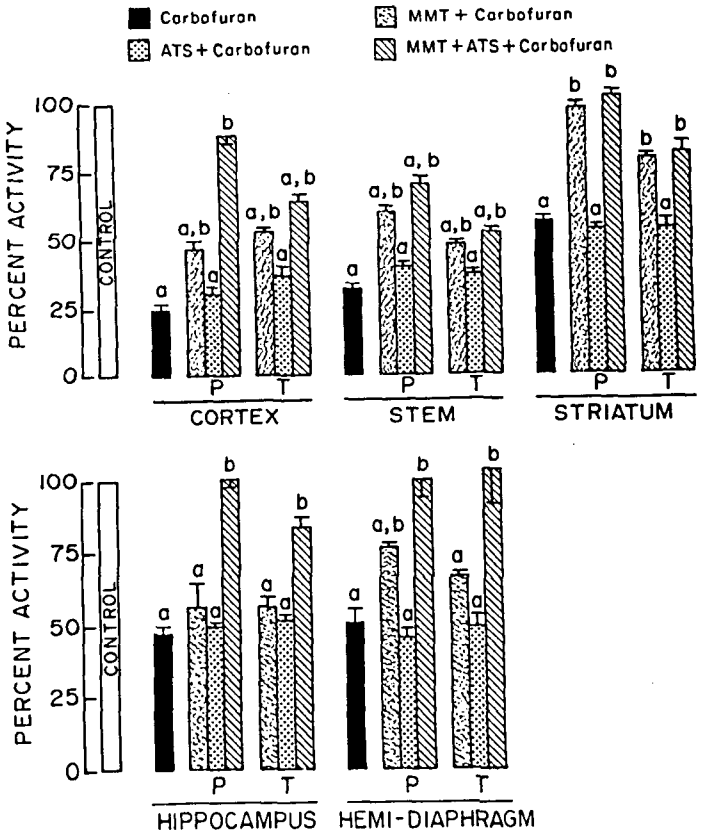


FIGURE 9. Effects of memantine hydrochloride (MMT, 18 mg/kg sc) and/or atropine sulfate (ATS, 16 mg/kg sc) given prophylactically (P) or therapeutically (T) on AChE activity (percent activity) in brain regions and hemidiaphragm of rats acutely intoxicated with carbofuran (1.5 mg/kg sc). a, Statistically significant difference between controls and treated rats ($p < .01$). b, Statistically significant difference between carbofuran and carbofuran plus antidote (MMT + ATS) treated rats ($p < .01$). From Gupta and Kadel (1989b).

including permeability (Gupta et al., 1993). Thus, several synaptic and nonsynaptic mechanisms of action of memantine at the cellular level could contribute to its efficacy against central and peripheral signs of carbofuran and other carbamates (Gupta & Kadel, 1989b, 1991a, 1991b).

Recently, Lotsev et al. (1990) reported reduced mortality in sheep intoxicated with carbofuran (3 mg/kg po) following atropine and diazepam treatment. Currently, prophylactic and therapeutic evaluation of glycopyrrolate against carbofuran poisoning is in progress (Gupta, unpublished observations).

RESIDUE ANALYSES OF CARBOFURAN AND ITS METABOLITES

Strenuous efforts have been made in the past in regard to residue analyses of carbofuran and its major metabolites (3-hydroxycarbofuran and 3-ketocar-

bofuran) in a variety of matrices. Several investigators have developed various radioactive and nonradioactive methods for the extraction, separation, identification, confirmation, and quantitation of carbofuran and its metabolites in animals, fish, insects, and plants (Dorough, 1968; Metcalf et al., 1968; Bruce, 1972; Williams & Brown, 1973; Wheeler et al., 1979a, 1979b; Sonobe et al., 1981, 1983; Splittler & Marafioti, 1983; Khan et al., 1987).

In 1976, Rangaswamy and his co-workers developed a spectrophotometric method for carbofuran analysis. Thereafter, for several years, gas chromatography (GC) was most commonly used for the determination of carbofuran and metabolites in human and animal fluids and tissues (Butler & McDonough, 1971; Wong & Fisher, 1975; Khan et al., 1987; Huang et al., 1989), plants (Nelson & Cook, 1980; Lee & Westcott, 1983; Leppert et al., 1983), and soil and water (Greenhalgh & Balanger, 1981; Leppert et al., 1983). Recently, HPLC methods have been widely used for residue (carbofuran/metabolites) determination in technical and formulated products (Keiser et al., 1983; Kikta, 1986), soil, water, and air (U.S. EPA, 1984; Bouchard, 1987; Mori et al., 1987; McGarvey, 1993), animal tissues (Ali, 1989; Ali et al., 1993a, 1993b), and plants (Lawrence & Leduc, 1978; Lee & Westcott, 1980; McGarvey, 1993; Ling et al., 1993). Today, gas chromatography-mass spectroscopy (GC/MS) or liquid chromatography-mass spectroscopy (LC/MS) is considered the most powerful tool for the quantitation and confirmation of carbofuran and its metabolites, even in very complex matrices (Osheim et al., 1985; Voyksner, 1987; Khan et al., 1987). A typical total ion chromatogram (TIC) and mass spectrum of carbofuran is shown in Figure 10 (Gupta, unpublished observations). Detailed information on various analytical methods and their sensitivity for the residue analysis of carbofuran and its metabolites is given in Table 5.

BIOMARKERS AND TOXICITY ASSESSMENT

The presence of carbofuran and/or its major metabolites (3-hydroxycarbofuran and 3-ketocarbofuran) in food, water, or air could be considered as the source of contamination. The presence of carbofuran or its metabolite(s) in the urine, feces, bile, or any other body tissue(s)/fluid(s) can be considered as a most specific biomarker of recent or ongoing exposure. The excretion of 3-hydroxycarbofuran has been found to be more associated with carbofuran exposure than 3-ketocarbofuran in urine. Levels of 3-hydroxycarbofuran in patients with acute carbofuran poisoning may increase and reach 80.64 ppb (Huang et al., 1989). Due to extensive and rapid metabolism of carbofuran, levels are not expected to persist in tissue or fluid for prolonged periods after exposure.

Monitoring of preexposure and postexposure levels of AChE in erythrocytes gives a good measure of the effect of carbamate exposure (He, 1993). Erythrocyte AChE is more sensitive to carbamate ChE inhibitors than plasma

ChE (Vandekar et al., 1971; Wills, 1972; WHO Task Group, 1986). Erythrocyte AChE measurement is also more specific, since it is found in both the peripheral and central nervous systems, and therefore may be a more sensitive biomarker of neurological effects. In a recent investigation, inhibition of blood AChE in workers involved in carbofuran formulation correlated well with an airborne carbofuran concentration of higher than 0.1 mg/m^3 (Huang et al., 1989). Hussain et al. (1990) also demonstrated significant inhibition of blood AChE in grain farmers exposed to carbofuran. Plasma ChE was not affected. In fact, plasma or serum ChE is not only less sensitive to inhibition by carbamates (see data in Table 3), but the activity can also be suppressed by a variety of diseases (Henry, 1984; Coye et al., 1986).

It needs to be emphasized that recovery from carbamate-induced ChE inhibition is much faster than that following OP exposure, because carbamylation of the enzyme is easily and rapidly reversed (Coye et al., 1986; also see Mechanisms section of this review). It has been recommended that if ChE activity is to be determined for assessing carbamates exposure, blood samples should be drawn within 4 h after exposure (WHO Task Group, 1986), and analytic methods should be selected to give a possibility of determining ChE activity immediately after blood sampling (WHO Study Group, 1982). Because of the rapid reversibility of carbamate-induced ChE inhibition, the 30% reduction in ChE activity can only be recommended as an individual health-based biological limit for occupational exposure if analyses

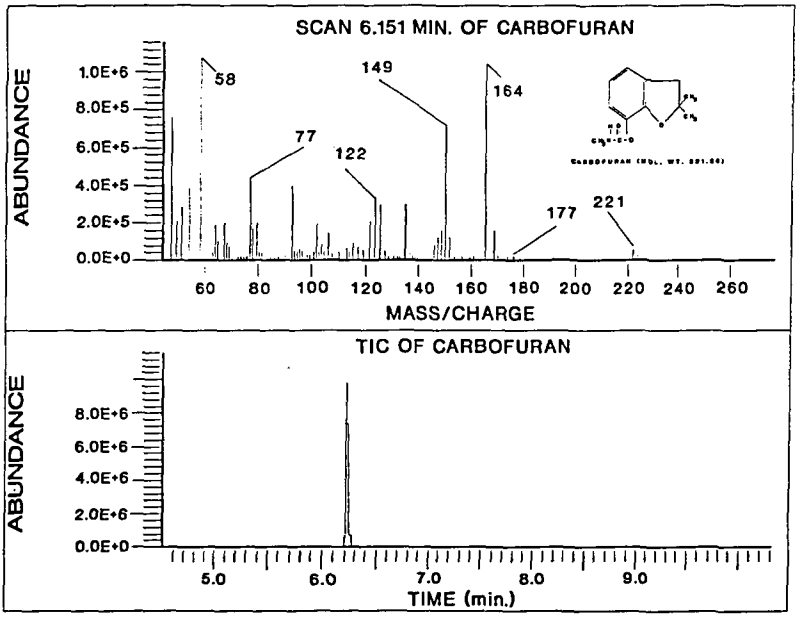


FIGURE 10. Total ion chromatogram (TIC, lower panel) and mass spectrum (upper panel) of carbofuran. Typical ions of carbofuran are 221, 164, 149, 122, 77, and 58.

TABLE 5. Analytical Methods and Their Detection Limits for Carbofuran and Its Metabolites in Various Matrices

Analytical method	Sample matrix	Detection limit	Reference
HPLC	Tap/well water	0.04–1 ppb	U.S. EPA (1984), Mori et al. (1987), Beauchamp et al. (1989), Corcia and Marchetti (1991), Edgell et al. (1991), Bushway et al. (1992)
	Wastewater	30 ppb	Bouchard (1987)
	Liver	5 ppb	Ali (1989), Ali et al. (1993a, 1993b)
	Urine	100 ppb	Hussain et al. (1990)
	Stomach/rumen content	5–10 ppb	Gupta (unpublished observations)
	Fruits/vegetables, crops	5–10 ppb	Lawrence and Leduc (1978), Lee and Westcott (1980), Krause (1985a, 1985b), Chaput (1988), Ling et al. (1993)
	Soil	20–100 ppb	Lauren (1984), Bouchard (1987), Mori et al. (1987)
GC	Water	2 ppb	Lancaster (unpublished observations)
		0.5 ppb	Weaver et al. (1983)
	Liver, kidney, muscle, eggs	50 ppb	Cook et al. (1977)
	Milk	20 ppb	Cook et al. (1977)
	Stomach/rumen content, feed	50 ppb	Stahr (1991)
	Urine	NS	Huang et al. (1989)
	Vegetables	100 ppb	Cook et al. (1977)
GC/MS	Rumen/stomach content, water	2 ppb	Gupta (unpublished observations)
LC/MS	Water, soil	0.1–1 ppb	Voyksner (1987)
FIAS	Sea water	6 ppb	Kumaran and Tran-Minh (1992)
EIA	Water	0.1 ppb	Bushway et al. (1992)
Radioactive	Milk	5–20 ppb	Ivie and Dorough (1968)
Spectrophotometric	Crops, soil	25 ppb	Rangaswamy et al. (1976)
Thin-layer chromatography	Stomach/rumen content	100 ppb	Stahr (1991)

Note. FIAS, flow injection analytical system; EIA, enzyme immunoassay; NS, not specified.

of blood samples can be carried out within the first few hours (WHO Study Group, 1982).

Thus, critical inhibition of AChE in blood, brain, or muscle, in humans, animals, birds, or fish, is strongly indicative of poisoning due to carbamate and/or OP. Cholinergic symptoms usually appear in carbamate-exposed workers with a blood AChE lower than 70% of the individual's baseline level (WHO Study Group, 1982; WHO Task Group, 1986; Huang et al., 1989; He, 1993). In interpreting results, it should be noted that human males have higher erythrocyte and plasma ChE levels than do females.

Analyses of serum enzymes (CK, LDH, and others) and their isoenzymes and subforms provide additional knowledge of tissue-specific damage (Gupta et al., 1991a, 1991b, 1993, 1994b) and can be considered as markers of effects. A serious limitation with AChE or any other biochemical markers is lack of specificity such that no marker indicates the presence of carbofuran or its metabolite(s).

While comparing the behavior and toxicity of carbofuran with its metabolites, using the microtox (bacterial testing) method for toxicity assessment, 3-hydroxycarbofuran was found less toxic than carbofuran, whereas 3-ketocarbofuran was found marginally more toxic than carbofuran (Kross et al., 1992). These studies suggest further evaluation of human and animal health effects of carbofuran metabolites, and warrant the attention of both the scientific community and regulatory agencies.

CARBOFURAN RESIDUE IN SAMPLES FROM THE ENVIRONMENT

As mentioned earlier, carbofuran is a rapidly degrading pesticide in the soil and water. Residue may persist in the soil and water, however, for several weeks or months (or up to a year, depending upon environmental conditions) (Finlayson et al., 1991; readers are also referred to the Metabolism section of this review).

Results from a study conducted by the Environmental Hazards Assessment Group of the California Department of Food and Agriculture reveal that runoff waters from rice and sugarbeet fields are potential sources of carbofuran found in agricultural drains and in the Sacramento River (Nicosia et al., 1990). Findings indicate that, of the two potential sources, runoff water discharged from rice fields contributed a much larger portion of carbofuran than runoff from sugarbeet fields. Concentrations of carbofuran were first measured in the agricultural drains in California in 1987 (Harrington & Lew, 1989). Maximum concentrations of carbofuran in the Colusa Basin Drain exceeded the criterion level (0.4 ppb), set for rice return water, in 1987 (13 ppb), 1988 (4.4 ppb), 1989 (1.5 ppb), 1990 (1.1 ppb), and 1991 (0.6 ppb). Similar data for the Sacramento River indicated that concentrations of carbofuran were detected up to 2.1 ppb in the river water near the city of Sacramento in 1987, but < 1 ppb since 1988 (Table 6).

Occasionally, the residue of carbofuran has been detected in water samples collected from the wells (Weaver et al., 1983; Bushway et al., 1992) and the spring runoff (Waite et al., 1992). No studies were located regarding monitoring of carbofuran residue in samples from the air or landfill sites.

EPILOGUE

Carbofuran is an extremely toxic carbamate pesticide and is widely used in agriculture and forestry. Its improper use often results in contamination of the environment and consequently in deleterious health effects. Malicious poisoning in animals and birds generally leads to death. In mammals, carbofuran rapidly metabolizes to the equally toxic metabolite 3-hydroxycarbofuran or to several less toxic metabolites. 3-Hydroxycarbofuran prolongs the toxicosis period by having an anticholinesterase property and by getting trapped in the enterohepatic cycle, even when the parent compound does not exist in the body. There seem to be minimal or no health risks to milk and meat consumers, since lactating animals when exposed to carbofuran

TABLE 6. Carbofuran Contamination in the Water, Soil, and Subsoil

Matrix	Location	Year	Residue level	Reference
Spring runoff	Saskatchewan, Canada	1987	1 ppb	Waite et al. (1992)
Well water	Maine, USA	1990	0.25–1.2 ppb	Bushway et al. (1992)
	Santa Ana, TX, USA	1982	0.5 ppb	Weaver et al. (1983)
Drain	Calusa Basin, CA, USA	1991	0.6 ppb	CDPR (1992)
		1990	1.1 ppb	Harrington and Lew (1992)
		1989	1.5 ppb	Harrington and Lew (1992)
		1988	4.4 ppb	Harrington and Lew (1989)
		1987	13.0 ppb	Harrington and Lew (1989)
River water	Sacramento River, CA, USA	1991	<0.4 ppb	CDPR (1992)
		1990	0.6 ppb	Harrington and Lew (1992)
		1989	<1.0 ppb	Harrington and Lew (1992)
		1988	<1.0 ppb	Harrington and Lew (1989)
		1987	2.1 ppb	Harrington and Lew (1989)

Note. CDPR, California Department of Pesticide Regulation.

through judicious use in agriculture largely excrete carbofuran and its metabolites in the urine, thereby leaving very little residue in the milk and tissues. However, in large doses carbofuran can produce a variety of toxicological effects. Exposure to carbofuran along with other anticholinesterase pesticide(s) may result in toxic potentiation, even when they are used in safe doses. Currently, antidotal treatment consisting of atropine and memantine is the most effective against acute poisoning of carbofuran.

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