CASE REPORT

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Poisoning from Oral Ingestion of Carbofuran (Furadan 4F), a Cholinesterase-Inhibiting Carbamate Insecticide, and Its Effects on Cholinesterase Activity in Various Biological Fluids

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ABSTRACT: A case is presented of a fatal ingestion of Furadan (carbofuran), a cholinesterase-inhibiting carbamate insecticide. A 26-year-old white male was found dead with a partially filled 1-gal (3.8-L) container of Furadan 4F insecticide-nematocide (44.9% carbofuran). The individual had ingested approximately 345 mL of the mixture. Analysis of cholinesterase activity in various biological fluids was performed spectrophotometrically using propionylthiocholine and 5,5′-dithiobis-2-nitrobenzoic acid [Sigma Diagnostics, cholinesterase procedure No. 422 (PTC)] which was measured at 405 nm and 30°C in a Gilford Stasar III Spectrophotometer. The cholinesterase activities were as follows: plasma, 245 units (U)/L (93% inhibition/7% normal activity); serum, 208 U/L (95.3% inhibition/4.7% normal activity); whole blood, 297 U/L (92.8% inhibition/7.2% normal activity); erythrocytes, 58 U/L (99% inhibition/1% normal activity); vitreous humor, 7 U/L; and bile, 148 U/L. Carbofuran was detected in the blood and gastric contents by thin-layer chromatography. No alcohol or other drugs were detected in the blood, urine, or gastric contents. Ingestion of the carbofuran produced acute visceral congestion and pulmonary edema. Death was caused by anoxia due to respiratory paralysis produced by cholinesterase inhibition from Furadan (carbofuran) ingestion.

KEYWORDS: toxicology, carbofuran, Furadan, insecticides, cholinesterase, carbamate insecticides, cholinesterase inhibition

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Carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranylmethylcarbamate) (Furadan) is a carbamate insecticide. It is found in a variety of commercially available veterinary and agricultural products. Carbofuran is considered one of the more highly toxic carbamate insecticides (Table 1). Carbofuran, like the organophosphates, inhibits cholinesterase, which produces excessive accumulation of acetylcholine at muscarinic, nicotinic, and central nervous system receptors. Toxic manifestations of this are diarrhea, urination, miosis, bronchospasm, emesis, lacrimation, salivation, muscle fasciculation, and convulsions [*1–3*]. Signs and symptoms of toxicity usually occur within 30 min but may develop up to 1 to 2 h after exposure [*2*]. The carbamates as a class are less toxic than the organophosphates, although their clinical effects are similar. Carbofuran can be absorbed by inhalation, by ingestion, and through the skin [*1–3*]. Diagnosis of carbofuran intoxication is based upon a history of exposure and characteristic development of a cholinergic symptomatology. Clinical manifestations of carbamate exposure are of a shorter duration than those of organophosphate exposure because carbamylation of the cholinesterases is more readily reversible than that of the phosphorylated cholinesterases from organophosphates [*1,3*]. Red blood cell and plasma acetylcholinesterase levels are generally not considered helpful, since carbamates only have transient effects on these activities [*1,2*].

Occupational exposures to carbofuran have produced measurable concentrations of carbofuran in biological fluids but no observed inhibition of cholinesterase activity and no acute adverse effects for up to four days after exposure when proper protective wear was used [*4*]. Agricultural exposure without protective wear has also been reported to produce signs of classical cholinesterase inhibition [*5*]. A fetal death was observed following carbamate poisoning of a pregnant woman [*6*].

This presentation is of a fatal ingestion of carbofuran. Carbofuran concentrations were determined in the biological fluids of the individual, as well as corresponding cholinesterase activities. Cholinesterase activity in vitreous humor and bile from normal individuals was studied to establish normative data in relation to which the degree of inhibition produced by the carbofuran overdose in this case was ascertained.

Case History

A 26-year-old while male (approximately 95 kg in weight) was found dead on a trail on Buffalo Mountain in Washington County, Tennessee. A .22 caliber rifle was found lying beside the victim, along with a 1-gal (3.8-L) white plastic container labeled Furadan

TABLE 1—*Carbamate insecticides.*[a]

Highly Toxic (LD_{50} < 50 mg/kg)[b]	Moderately Toxic (LD_{50} > 50 mg/kg)	Low Toxicity (LD_{50} > 1g/kg)
aldicarb	dioxacarb	metam sodium
oxamyl	bendiocarb	
carbofuran	promecarb	
methomyl	bufencarb	
formetanate	MTMC (Metacrate)	
aminocarb	propoxur	
dimetilan	pirimicarb	
	MPMC (Meobal)	
	isoprocarb	
	carbaryl	

[a]Ref *2*.
[b]LD_{50} = median lethal dose.

4F insecticide-nematocide (44.9% carbofuran), of which 345 mL was unaccounted for. The deceased had been reported missing the day before and had stated he was going on a hunting trip. A history of attempted suicide had been reported.

Pathological Findings

External examination revealed that a white powdery substance (carbofuran) was present in and about the nose and mouth as well as on the hands and clothing of the deceased. The finely particulate white powder was present within the oral pharynx and over the tongue without overt hemorrhage or ulceration. No large foreign bodies were found in the upper airways. Slight hyperemia was present on the lips, which was associated with the white powder. Histological examination revealed acute visceral congestion and pulmonary edema. Incidental findings included an unruptured venous angioma in the right temporal lobe, mild hepatic steatosis, and an anomalous origin of the left common carotid artery from the brachiocephalic trunk.

Materials and Methods

Specimen Collection

Blood (60 mL) was collected by needle aspiration from the subclavian vein and stored in six (10-mL) vacutainer tubes: two of the tubes were sterile, two tubes contained potassium ethylenediaminetetraacetate (K_3EDTA), and two tubes contained sodium fluoride and potassium oxalate. Vitreous humor was collected by needle puncture of the posterior chambers of both eyes and stored in a sterile vacutainer tube. Bile and urine were collected by bladder punctures and stored in plastic specimen containers. Gastric contents (100 mL) were collected and stored in a plastic specimen container. Plasma and serum were separated by centrifugation at 2000 rpm for 10 min. The biological fluids were stored at 2 to 4°C until analysis.

Bile and vitreous humor specimens were collected from 18 other autopsies in which the individuals had died either from natural causes or from fatal gunshot wounds; no drugs or toxins were detected in these specimens. The specimens were used to establish normative cholinesterase activity data for these body fluids in order to determine the degree of inhibition produced in this case.

Analytical Methods

The blood and urine specimens were tested for ethanol using the Abbott TDx/radiative energy attenuation (REA) ethanol assay (Abbott Laboratories, North Chicago, Illinois). The ethanol concentrations were determined by gas-liquid chromatography [7]. The biological fluids and gastric contents were analyzed for numerous acidic, basic, and neutral drugs and metabolites—including narcotics and other analgesics, barbiturates and other sedative hypnotics, benzodiazepines, cannabinoids, cocaine, phencyclidine, phenothiazines, sympathomimetic amines, and tricyclic antidepressants—by a combination of thin-layer chromatography (TLC), (Toxi-Lab System, Toxi-Lab Inc., Irvine, California), gas chromatography (GC), gas chromatography/mass spectrometry (GC/MS), enzyme-multiplied immunoassay technique (EMIT), fluorescence polarized immunoassay (FPIA), and specific colorimetric procedures.

Carbofuran was extracted from the biological specimens at pH 9 using Toxi-Tube A. TLC analysis of carbofuran was achieved using Toxi-Grams A glass microfiber paper chromatograms with silicic acid. Toxic-Grams A chromatograms impregnated with vanadium salt were developed using 3 mL of ethyl acetate/methanol/water (87:3:1.5) with

15 μL of ammonium hydroxide (28% NH_4OH). The chromatograms were developed to 10 cm. The developed chromatograms were dried with heat, and detection was achieved by dipping the chromatograms into various chromogenic reagents. The chromogenic procedures for the Toxi-Grams A chromatograms included exposure to formaldehyde vapor, sulfuric acid (concentrated), water, ultraviolet (UV) light (long wave, 366 nm), and modified Dragendorff's reagent.

Carbofuran confirmation and quantitation were conducted on a Perkin-Elmer Model 8500 gas chromatograph (Perkin-Elmer, Norwalk, Connecticut), equipped with a flame ionization detector and a 6-ft (1.8-m) by 2-mm (inside diameter) glass column packed with 3% SP2250DA on 100/120 mesh Supelcoport (Supelco, Bellefonte, Pennsylvania). The chromatographic conditions were the following: injection temperature, 240°C; column temperature program, 150°C (held for 1 min) raised to 200°C at 10°C/min, then to 200 to 240°C at 20°C/min, and held at 240°C (for 1 min); flame ionization detector temperature, 300°C; and helium as the carrier gas, at a flow rate of 50 mL/min. The chromatograms were recorded and integrated using a Hewlett-Packard 3390A integrating recorder (Hewlett-Packard, Richardson, Texas). Carbofuran was extracted from biological fluids (1 mL of specimen to 4 mL of water) spiked with aprobarbital (20 μg) as an internal standard, using a Toxi-Tube B at pH 4.5. The organic extracts were removed and evaporated to dryness at 45°C under a stream of nitrogen. The residues were reconstituted with 20 μL of methanol and 2 μL of the reconstituted residue were injected for analysis.

Cholinesterase activity was determined in the various biological fluids spectrophotometrically using the Ellman reaction [8] as follows:

$$\text{Propionylthiocholine} + H_2O \xrightarrow{E} \text{propionic acid} + \text{thiocholine}$$

$$E = \text{acetylcholinesterase}$$

$$\text{Thiocholine} + \text{5,5'-dithiobis-2-nitrobenzoic acid} \rightarrow \text{5-thio-2-nitrobenzoic acid}$$

The activity was measured at 405 nm and 30°C in a Gilford Stasar III spectrophotometer (Gilford Medical Instruments, Berlin, Ohio) using Sigma Diagnostics cholinesterase procedure No. 422 (PTC) (Sigma Diagnostics, St. Louis, Missouri).

Results

Carbofuran was identified in the blood and gastric contents of the victim by TLC. Carbofuran was extracted and migrated to an Rf of 0.95 with the Toxi-Lab A system. It produced a purple-centered spot with a pink halo on reaction with Mandelin's reagent; produced a gray spot with a purple center after contact with water and heat generation; absorbed UV light and was stained dark brown with Dragendorff's reagent. Carbofuran was confirmed and quantitated by gas chromatography. It eluted in 6.08 min with a retention index of 1.33 relative to aprobarbital. Typical chromatograms are illustrated in Fig. 1. The blood carbofuran concentration was determined to be 29.3 μg/mL. The deceased had ingested approximately 345 mL of the Furandan 4F mixture (44.9%) or 155 g of carbofuran (approximately 1.6 g/kg of body weight), of which we found 50.6 g still remaining in the gastric contents.

The high concentration of carbofuran in the body did produce substantial cholinesterase inhibition. The cholinesterase activity in the biological fluids is given in Table 2. The plasma, serum, erythrocyte, and whole blood cholinesterase activities were all greater than 92% inhibited. The lack of normative cholinesterase activity data for the vitreous humor and bile initially prevented determination of the degree of cholinesterase inhibition

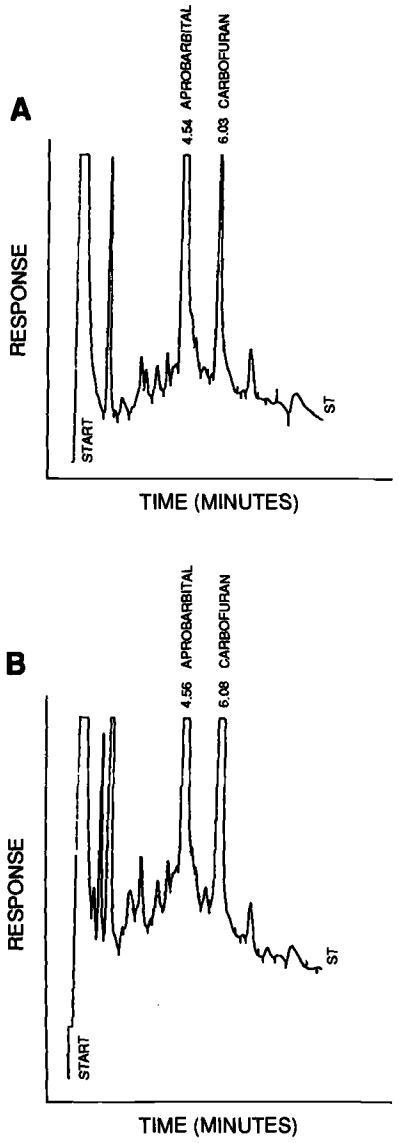

FIG. 1—*Chromatograms of carbofuran and aprobarbital from plasma:* (a) *typical plasma specimen* (b) *plasma standard with 20 μg/mL carbofuran.*

in these fluids by carbofuran. The results of cholinesterase activity determinations in vitreous humor and bile from the 18 control autopsies are given in Table 3. Cholinesterase activity was inhibited by carbofuran 87% in the vitreous humor and 74% in the bile, based on these control data.

Discussion and Conclusions

The mechanism of toxicity for carbofuran is inhibition of cholinesterase, which produces signs and symptoms of cholinergic excess, typically, salivation, lacrimation, miosis, res-

TABLE 2—*Cholinesterase activity in various biological fluids.*

Specimen	Cholinesterase Activity, U/L	Inhibition, %	Normal Activity, %	Normal Activity Range, U/L
Plasma	245	93	7	1700 to 4100
Serum	208	95	5	3100 to 7700
Whole blood	297	93	7	3300 to 5500
Erythrocytes	58	99	1	4400 to 8200
Vitreous humor	7	87	13	15 to 163
Bile	148	74	26	297 to 891

TABLE 3—*Normative cholinesterase activity, in units per litre, in vitreous humor and bile.*[a]

Specimen	*n*	Mean	SD	SE
Vitreous humor	18	54	32	7.6
Bile	14	573	183	48.9

[a]Key to abbreviations:
n = number of observations.
SD = standard deviation.
SE = standard error of the mean.

piratory paralysis, convulsions, and death [*3*]. Carbamates are relatively rapidly reversible inhibitors of cholinesterase because of in vivo spontaneous hydrolysis of the carbamylated acetylcholinesterase, which leads to less severe symptoms of shorter duration. Penetration of the blood-brain barrier by the carbamates is poor; thus, they produce minimal effects on brain cholinesterase activity and few central nervous system symptoms [*1*]. Cholinesterase levels are generally not helpful in determining the etiology of the toxin since carbamates only have transient effects (1 to 2 h) at these levels [*1–3*].

This scenario is true in most situations of acutely toxic, sublethal exposures to many of the less potent carbamates, but the situation is different when a large quantity of carbofuran or one of the highly toxic carbamates is ingested as in the case of an overdose. The individual reported on may have ingested up to 155 g of carbofuran, of which 50.6 g remained in his stomach. The plasma, serum, erythrocyte, and whole blood cholinesterase activities in this case revealed extreme inhibition (>92%). The excessive concentration of carbofuran in the blood (29.3 μg/mL) maintained a persistent carbamylation of the cholinesterase, regardless of any in vivo hydrolysis that might have occurred. The decreased inhibition of cholinesterase activity in the vitreous humor and bile is attributable to decreased penetration of tissue barriers. All levels of cholinesterase inhibition found in this case are considered lethal.

The concentration of carbofuran observed was more than ten times greater than the maternal blood concentration of 2.6 μg/g reported by Klys et al. [*6*] to have produced fetal death with in utero exposure. The estimated dosage of 155 (approximately 1.6 g/kg of body weight) or even the dosage based upon the amount of carbofuran remaining in the gastric contents (50.6 g, approximately 0.5 g/kg of body weight far exceeds the human oral median lethal dose (LD_{50}) of 11 mg/kg [*9*].

The pathological findings, combined with the toxicological results, substantiate that the victim died of anoxia due to respiratory paralysis produced by cholinesterase inhibition, as a result of carbofuran ingestion. The manner of death was reported to be suicide.

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References

[*1*] Ellenhorn, M. J. and Barceloux, D. G., *Medical Toxicology: Diagnosis and Treatment of Human Poisoning*, Elsevier, New York, 1988, pp. 1069–1078.

[*2*] Olson, K. R., *Poisoning and Drug Overdose*, Appleton and Lange, Norwalk, CT, 1990, pp. 106–108.

[3] Murphy, S. D., "Toxic Effects of Pesticides," *Cassarett and Doull's Toxicology: The Basic Science of Poisons*, 3rd ed., C. D. Klaassen, M. O. Andur, and J. Doull, Eds., Macmillan, New York, 1986, pp. 519–543.
[4] Hussain, M., Yoshida, K., Atrimo, M., and Johnston, D., "Occupational Exposure of Grain Farmers to Carbofuran," *Archives of Environmental Contamination and Toxicology*, Vol. 19, 1990, pp. 197–204.
[5] Coleman, A. M., Smith, A., and Watson, L., "Occupational Carbamate Pesticide Intoxication in Three Farm Workers," *West Indian Medical Journal*, Vol. 39, 1990, pp. 109–113.
[6] Klys, M., Kosum, J., Pach, J., and Kamenczak, A., "Carbofuran Poisoning of a Pregnant Woman and Fetus per Ingestion," *Journal of Forensic Sciences*, Vol. 34, 1989, pp. 1413–1416.
[7] Manno, B. R. and Manno, J. E., "A Simple Approach to Gas Chromatographic Microanalysis of Alcohols in Blood and Urine by a Direct-Injection Technique," *Journal of Analytical Toxicology*, Vol. 2, 1978, pp. 257–261.
[8] Dietz, A. A., Rubeinstein, H. M., and Lubrano, T., "Colorimetric Determination of Serum Cholinesterase and Its Genetic Variants by the Propionyl-Thiocholine-Dithiokis [Nitrobenzoic Acid] Procedure," *Clinical Chemistry*, Vol. 19, 1973, pp. 1309–1313.
[9] Sax, N. I., Ed., *Dangerous Properties of Industrial Materials*, 6th ed., Van Nostrand Reinhold, New York, 1984, pp. 1152–1153.

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