ıma, S., and Gelboin, H. V. 1978. Sulfate conjugation of benzo(a)pyrene derivatives. *Chem.-Biol. Interact.* 23:19–30.
F. J. C., and Warwick, G. P. 1969. Elimination of radioactivity after tillation of tritiated 3,4-benzopyrene in hamsters. *Br. J. Cancer* 254:103–115.
perimental study of hypersecretion of mucus in the bronchial tree. *Br. J. Exp.* 145.
S. A., Grote, M. I., and Kork, D. B. 1964. Retention rates of particulate e lungs. *Chicago Med. School Q.* 24:10–17.
F., and Kolb, L. H. 1968. A method for the experimental induction of cinoma. *Cancer Res.* 28:104–124.
no, R., Sellakumar, A., Cefis, F., and Kaufman, D. G. 1972. Respiratory tract hamsters induced by different numbers of administrations of benzo(a)pyrene *Cancer Res.* 32:1073–1081.
W. C., Hauser, R., Fox, F. T., and Stanley, T. W. 1960. Benzo(a)pyrene r of American communities. *Am. Ind. Hyg. Assoc. J.* 21:443–451.
T. R., and Stanley, T. W. 1966. Ultraviolet, visible and fluorescence spectral uclear hydrocarbons. *Int. J. Air Pollut.* 2:253–272.
ıwland, J. 1978. Role of particle size in the formation of respiratory tract ıy benzo(a)pyrene. *Eur. J. Cancer* 14:321–326.
rotection Agency. 1974. Health consequences of sulfur-oxides—A report from 71. EPA publication no. EPA 650/1-74-004.
Stereological Methods. Practical Methods for Biological Morphometry, vol. I. ic.
ıer, A. P., Charlson, R. J., and Ahlquist, N. C. 1977. Sulfate aerosol: Its nt in the midwestern and southern United States. *Science* 195:979–980.
N., and Koyama, H. 1972. Spontaneous tumors in hamsters: Incidence, splantation, and virus studies. *Gann* 63:329–336.
, J., and Gelboin, H. V. 1978. Benzo(a)pyrene metabolism: Activation and ı *Polycyclic Hydrocarbons and Cancer*, eds. H. V. Gelboin and P. O. P. Tso, 31. New York: Academic.

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A FARMWORKER DEATH DUE TO PESTICIDE TOXICITY: A CASE REPORT

Marshall H. Lee

Department of Community Health, Northern California Occupational Health Center, University of California, Davis, Davis, California

James F. Ransdell

Consulting Pathologist, Yolo County, Davis, California

In the state of California, the use of pesticides is closely regulated. Physicians are r quired to report all occupational illness attributable to pesticide use. The case of a deat involving the handling of aldicarb, a potent methylcarbamate insecticide, is presente Although the autopsy indicated trauma as the cause of death, subsequent analysis c tissue residues of aldicarb and its metabolites suggested that pesticide toxicity may hav been a contributing cause.

INTRODUCTION

A large proportion of deaths attributable to pesticides is due to accidental or intentional (such as suicidal) exposure to chemicals used in the control of weeds, insects, and plant disease in agricultural production. The last agricultural fatality due to pesticide use in the field was reported in 1972, occurring as a result of exposure to parathion, propargite, and potassium nitrite. Other deaths due to fumigation of buildings with cyanide and to handling and storage of ethylene dibromide (California Department of Food and Agriculture, 1983) have been recorded since that time.

Of the approximately 1400 chemicals whose use is monitored by the California Department of Food and Agriculture, aldicarb, a pesticide of the *N*-methylcarbamate group, is considered one of the most toxic substances of its class (California Department of Food and Agriculture, 1981). In order to reduce its tendency to disperse in air as dusty particulates, which can stick to wet skin, aldicarb is now available commercially only in the form of granules. One of the most widely used aldicarb formulations, Temik (Union Carbide), contains 10% (Temik 10G) or 15% (Temik 15G) aldicarb by dry

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veight. In 1982, the use of 200,995 pounds of active ingredient aldicarb, a ubstantial portion of which was formulated as Temik, was reported to the California Department of Food and Agriculture (1982). Temik is applied o the soil and can act as a systemic insecticide when plant uptake occurs. t has been used to control insects, mites, and nematodes on cotton, sugar eets, potatoes, peanuts, ornamentals, and sweet potatoes (Meister et al., 978). Since dissolving the granulates in water results in a highly hazardous olution, aldicarb formulations are registered to be used only for soil appli- ation.

Only one death, which occurred in 1969, has been attributed to aldicarb ince the inception of the California occupational health data system in 950 (California Department of Public Health, 1969). The case presented in his report is a recent death of an agricultural worker during the loading of ranulated aldicarb formulation.

REPORT OF A CASE

A 20-yr-old Mexican male was hired originally as an irrigator on a large ugarbeet farm. On the morning of May 14, 1982, he was assigned to load ranulated aldicarb, formulated as Temik 15G, into a hopper. This was pparently his first assignment as a pesticide loader. After having emptied n undetermined number of Temik bags, he was observed by an overseer o be lacking protective equipment, and was subsequently given an organic apor respirator, goggles, and paper coveralls. He returned to work without ny observable signs or symptoms. A second man, operating the tractor- lriven soil injection device, returned periodically to have his emptied hopper efilled by the first worker at the loading site, who may have opened and mptied 14 bags of Temik. About 200 ft from the loading site, the emptied emik packaging was burned. A third man, driving an eight-wheeled articu- ated tractor towing a disc harrow, complete his work in a field adjacent to he loading site, and was in motion along its border when he stopped to void irrigation equipment. He discovered a body on the ground between is tractor and the trailing disc blades, obviously crushed by the right rear et of wheels. Neither he nor anyone else witnessed the actual event, which ccurred about 2 h after the victim had begun to load Temik. After on-site esuscitation attempts failed, the victim's body was transported to a local mergency room, where he was declared dead on arrival. The body was emoved to the Coroner's facility, where the initial postmortem procedures vere performed without the knowledge that the victim had been working vith a pesticide. At autopsy, multiple trauma was evident. There were three ompound fractures of the left lower extremity. A subgaleal hematoma was oted in the left parietal region, with no associated skull fracture or under- ying cerebral injury. The thoracic cavity was crushed, and multiple pul- nonary lacerations were observed with no evidence of thoracocutaneous enetration. A massive left hemothorax was present. A superficial laceration

that did not penetrate the abdominal cavity was noted in the right inguinal region. The immediate cause of death was determined to be massive crushing chest injuries.

Blood was obtained for toxicological examination, which revealed no detectable levels of alcohol, barbiturates, hypnotics, tranquilizers, or salicylate. A single blood sample was preserved in a new, chemically clean collection bottle with sodium fluoride prior to receipt of information indicating the potential involvement of a pesticide, and there was no subsequent opportunity to obtain further blood samples. Michel technique (1949) red cell cholinesterase activity was determined to be 0.37 delta pH units (laboratory normal, 0.55–1.25 delta pH units).

Following the belated arrival of information indicating possible pesticide exposure, samples of the victim's liver, one kidney, and skin, including dermis and subcutaneous fat from the dorsum of both hands, anterior abdomen, and both thighs, were obtained for residue analysis. Vitreous humor was aspirated from both eyes and heparinized. Articles of the victim's clothing and protective gear were also collected for residue analysis.

METHODS

The tissue samples were extracted according to a modification of a Union Carbide method (1981a, 1981b), and residues of aldicarb and its chief metabolites, aldicarb sulfoxide and aldicarb sulfone, were quantitated using high-performance liquid chromatography (HPLC) and an ultraviolet fluorescence technique. Clothing samples were washed according to the Worker Health and Safety Dislodgeable Residue Procedure, and the aqueous solution was subjected to resin (Amberlite XAD-2) extraction (Sundaram et al., 1979). Aldicarb and aldicarb metabolite residues were then eluted from the resin column with an organic solvent and quantitated using HPLC with ultraviolet fluorescence detection employing postcolumn derivatization of the residues.

RESULTS

The results of tissue analysis are illustrated in Table 1; clothing residues are detailed in Table 2. Cholinesterase activity assay subsequently performed on the vitreous humor was reported as zero delta pH units (no activity). Sodium fluoride, a commonly used anticoagulant and preservative in blood tests, is also a known inhibitor of cholinesterase activity (Heilbronn, 1965). In the case reported here, the inadvertent use of fluoride makes the reported blood value invalid.

The active ingredient in Temik is aldicarb. Although there is no evidence that spontaneous oxidation in the formulated product yields significant levels of sulfoxide or sulfone, this worker's clothing was contaminated with aldicarb sulfoxide ranging from 2 to 37% of total residues. Sulfone was not detected on the clothing, however. Therefore, we assume primary exposure

TABLE 1. Residue of Aldicarb and Metabolites in Body Tissues (ppm)

Tissue	Aldicarb	Aldicarb sulfoxide	Aldicarb sulfone	Aldicarb derivatives total
Blood	MDL[a]	0.108	0.374	0.482
Liver	0.013	0.058	0.116	0.187
Kidney	Trace[b]	0.261	0.422	0.683
Skin (hand)	0.492	0.157	0.174	0.823
Skin (abdomen)	0.005	0.015	Trace[c]	0.020
Skin (thigh)	0.168	0.126	0.083	0.377

[a] Minimal detectable level = 0.028 ppm.
[b] Less than 0.002 ppm.
[c] Minimal detectable level = 0.024 ppm.

in this case was to aldicarb and its sulfoxide, whose toxicities are comparable. The oxidation of one molecule of aldicarb yields one molecule of the sulfoxide, whose oxidation in turn yields one molecule of aldicarb sulfone. Therefore, the sum of all detectable tissue aldicarb residues, including the parent compound and the metabolic products of the two-step oxidative process, should represent an approximation of the initial aldicarb "dose." This is illustrated by the rightmost column of Table 1. In this case, detectable levels of total aldicarb derivatives were found in all tissues analyzed. In blood, no aldicarb was detected, and aldicarb sulfone constituted 76% of the total aldicarb found, indicating the completeness of the two-step oxidative process in the circulation by the time of analysis. Aldicarb metabolites also comprised 40% of the total aldicarb found on the skin of the hand, a presumed exposure site. The high total aldicarb concentration found in renal tissue is evidence that excretion was already occurring at the time of death.

We then estimated the body burden of aldicarb based on total body water distribution. Aldicarb is relatively lipophilic (benzene : water solubility coefficient of 24 at 30°C), and is a small molecule with a relatively simple

TABLE 2. Residues of Aldicarb and Metabolites on Clothing (mg)

Clothing	Aldicarb	Aldicarb sulfoxide	Aldicarb sulfone	Aldicarb derivatives ($\mu g/m^2$)
Jacket lining	516.2	53.0	ND[a]	1,355
Jacket collar	169.4	30.3	ND	4,993
Pants	409.6	136.4	ND	475
Slipper	148.4	13.9	ND	5,976
Socks (2)	308.1	7.5	ND	1,262
Rubber glove	548.4	44.2	ND	15,595
Coveralls	11,129.0	271.5	ND	6,706
Shirt	258.1	151.9	ND	342
Elastic strap	124.2	69.4	ND	14,900

[a] Not detectable at minimum detectable level = 5.5 μg/sample.

configuration. Thus, limiting our calculation to a volume of distribution equalling total body water should be very conservative, since lipophilic substances typically have volumes of distribution greater than total body water. We obtain a total body burden of 18.2 mg [Eq. (1)]. By dividing this figure by the victim's body weight (66.28 kg), we obtain 0.275 mg/kg as the "dose." Our calculated dose appears to exceed the oral dose (0.1 mg/kg) shown to produce cholinergic effects in humans (Cope and Romine, 1973).

$$\text{Aldicarb equivalents measured in blood} = 0.482 \text{ ppm}$$

$$\text{Total body water, in mg, (57\% of body weight)} = 37.78 \times 10^6 \text{ mg}$$

$$\text{Body burden of aldicarb based on total body water distribution}$$

$$= (0.482 \text{ ppm})(37.78 \times 10^6 \text{ mg}) = 18.2 \text{ mg} \quad (1)$$

The vitreous humor is less susceptible to rapid chemical changes than blood after death. Vitreous fluid has been used for chemical assays to establish possible cause and time of death (Coe, 1969), especially where traumatic injury has contaminated blood, such as by rupture of abdominal organs, or in cases where exsanguination makes postmortem blood unavailable. In this case, the vitreous material was devoid of cholinesterase activity. There are no previous reports of cholinesterase activity assay in vitreous material. In two other routine autopsies performed by one of the authors (JFR), simultaneous red cell and vitreous cholinesterase activity assays indicate that some level of enzyme activity is present in the vitreous fluid.

DISCUSSION

Although the death of this worker was directly attributable to massive chest trauma, the circumstances surrounding the accident and the results of toxicological analysis indicate that pesticide intoxication played a contributory role. The victim was found to be wearing fleece-lined slippers, footwear appropriate only for household use. His lunch was found near the accident site, but no facilities for washing nor sources of potable water were available. The victim had not undergone baseline cholinesterase testing as required by worker protection statutes (California Administrative Code, 1982) prior to his job assignment with a highly toxic cholinesterase-inhibiting compound.

In a human experiment conducted in 1970, the manufacturer of Temik reported cholinergic symptoms in men who ingested doses of 0.1 mg/kg (Cope and Romine, 1973; Haines and Heywood, 1971). Weakness in the extremities, speech slurring, diaphoresis, and pupillary mydriasis were noted, and in one case, nausea and vomiting occurred. In men ingesting 0.1 mg aldicarb/kg, fast radiometric whole-blood cholinesterase activity assay revealed significant enzymatic depression to a mean of 28.1% of predose

activity (at 18 h predose) within 2 of ingesting aldicarb (mean 2-h cholinesterase depression significantly different from predose at $p < 0.01$ by paired t-test). No symptoms were observed in men given aldicarb at 0.05-mg/kg and 0.025-mg/kg doses. In men given the highest oral dose, symptoms had resolved by the 4th postdose hour. These observations indicate the rapidity of symptom onset (and also resolution) in toxic exposures to aldicarb.

No blood levels of aldicarb or its metabolites were obtained in these experiments. We can find no data on the volume of distribution of aldicarb. If we accept the conservative volume of distribution equal to total body water, the plasma concentration at time zero of a 0.1 mg/kg dose would be calculated as follows:

$$\text{Volume of distribution (Vd)} = \frac{\text{dose}}{\text{plasma concentration (Cp)}}$$

or

$$Cp = \frac{\text{dose}}{Vd}$$

For a 66.28-kg man, then

$$Cp = \frac{(0.1 \text{ mg/kg})(66.28 \text{ kg})}{(57\%)(66.28 \text{ kg})}$$

with a resulting plasma concentration of 0.175 mg/kg (0.175 mg/l). If the volume of distribution is greater, as one would expect from the chemical characteristics of aldicarb, the plasma concentration that results from a 0.1-mg/kg dose would be smaller. Thus, even at the smallest Vd, the maximum estimated plasma concentration resulting from a dose known to cause symptoms in a man is only 36% of that of the plasma concentration actually measured in the victim (0.482 mg/l). We have ignored plasma half-life, which is necessary to arrive at a true estimate of the victim's initial exposure. Since we can find no specific data on either Vd or half-life of aldicarb, further calculations are at best speculative. In general, one may expect that the measured concentration of aldicarb products in the victim underestimated the concentration reached immediately after his initial "dose."

Dermal exposure is presumed to have been the victim's most important source of tissue aldicarb, and aldicarb concentration was highest in the skin of the hand. Levels in abdominal skin were far below the concentration found in the skin of the supposedly glove-protected hand. Since the glove was made of an impervious acrylic material, it is likely that the victim's hands were unprotected during at least part of the time he was loading aldicarb. Alternatively, he may have been wearing the gloves but permitted the granules to enter beneath them through the tops, leading to gross hand contamination.

Another possible route of aldicarb absorption is suggested by the victim's burning of the emptied Temik packaging. The volatility of aldicarb at high temperatures short of pyrolysis has not been extensively studied, although it is known that burning Temik results in production of toxic gaseous products, such as sulfur dioxide, which are not specific acetylcholinesterase inhibitors (Kennedy et al., 1978).

Aldicarb residues found on the victim's clothes appear to reflect the expected pattern, that is, highest concentrations were found on external apparel such as the gloves and coveralls. It is certain that some physical disloding of Temik residues from outer garments occurred during rescue and transport procedures on the victim, and no valid estimation from residue analysis on clothing can be made of the amount of Temik absorbed from the victim's immediate environment.

Factors that may have artifactually elevated tissue aldicarb concentrations were not observed. These include collection of coagulated blood, allowing evaporation of tissue samples, and contamination by external sources of Temik in postmortem procedures. Factors that may have artifactually lowered tissue aldicarb include postmortem degradation of aldicarb residues occurring in the interval between sample collection and analysis. Since tissue samples were refrigerated but not frozen for approximately 10 d, some hydrolysis and enzymatic loss most likely occurred (Andrawes et al., 1967). This further suggests that the measured residues may in fact have underestimated the total aldicarb present in the victim's body at the time of death.

It is evident from the toxicological studies that the victim absorbed a quantity of aldicarb as a result result of his occupational function. His choice of work apparel and the evidence of contamination of his hands indicated that he had, at best, a poor understanding of the safety precautions needed in handling this substance. While not specifically prohibited by worker safety regulations, the burning of the packaging material may have been another avoidable hazard. The victim had evidence of blood and tissue aldicarb at levels exceeding by a factor of nearly 3 that known to produce cholinergic symptoms in man. The protective measures and equipment he used were evidently inadequate. Physical incapacitation may have occurred to the extent that he was unable to perform the coordinated voluntary neuromuscular actions that could have prevented his death.

REFERENCES

Andrawes, N. R., Dorough, H. W., and Lindquist, D. A. 1967. Degradation and elimination of Temik in rats. *J. Econ. Entomol.* 60:979-987.

California Administrative Code. 1982. Title 3, Section 2477(4)(f).

California Department of Food and Agriculture. 1981. Pesticide Safety Information Series. HS-641(a). October 13.

California Department of Food and Agriculture. 1982. Pesticide report. Annual.

California Department of Food and Agriculture. 1983. Deaths due to exposure to pesticides reported to the California Department of Food and Agriculture between January 1 and December 31, 1982. HS-1102.

California Department of Public Health. 1969. Occupational diseases in California attributed to pesticides and other agriculture chemicals, p. 13.

Coe, J. I. 1969. Postmortem chemistries on human vitreous humor. *Am. J. Clin. Pathol.* 51:741–750.

Cope, R. W. and Romine, R. R. 1973. Temik 10G aldicarb pesticide. Results of aldicarb ingestion and exposure in working environments. Project report, Union Carbide Corporation.

Haines, R. G. and Heywood, D. L. 1971. Ingestion of aldicarb by human volunteers: A controlled study of the effect of aldicarb on men. Union Carbide Corporation.

Heilbronn, E. 1965. Action of fluoride on cholinesterase. I. On the mechanism of inhibition. *Acta Chem. Scand.* 19:1333–1346.

Kennedy, M. V., Holloman, M. E., and Hutton, F. Y. 1978. Thermal degradation of selected fungicides and insecticides. *ACS Symp. Ser. (Disposal and Decontamination of Pesticides)* 81:99.

Meister, R. T., Berg, G. L., Sine, C., Meister, S., eds. 1983. Temik. In *Farm Chemicals Handbook*. C-230. Willoughby, Ohio: Meister.

Michel, H. O. 1949. Electrometric method for determination of red blood cell and plasma cholinesterase activity. *J. Lab. Clin. Med.* 34:1564–1568.

Sundaram, K. M. S., Szeto, S. Y., and Hindle, R. 1979. Evaluation of Amberlite XAD-2 as the extractant for carbamate insecticides from natural water. *J. Chromatog.* 177:29–34.

Union Carbide Corporation. 1981a. Union Carbide method UC 21140-III. Blood. Determination of total toxic Temik residues in beef liver by gas chromatography.

Union Carbide Corporation. 1981b. Union Carbide method. Aldicarb. FPD-liver (a). Jacksonville, Fla.

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NEUROBEHAVIORAL ASSESSMENT OF CHRONIC LOW-LEVEL METHYL BROMIDE EXPOSURE IN THE RABBIT

J. M. Russo, W. K. Anger, J. V. Setzer, W. S. Brightwell

National Institute for Occupational Safety and Health,
Division of Biomedical and Behavioral Science,
Cincinnati, Ohio

The research reported here was intended to identify the concentration at which methyl bromide begins to produce neurotoxic effects in the rabbit, a species known to be sensitive to this compound. Rabbits were exposed via inhalation to 27 ppm methyl bromide over a period of 8 mo for a total exposure duration of 900 h. Biweekly neurobehavioral tests, consisting of the latency rates of the ulnar and sciatic nerves and the amplitude of the eyeblink reflex of the orbicularis oculi muscle, failed to uncover any untoward consequences of the exposures. The rabbits gained weight and otherwise appeared to be healthy. In contrast to reports available in the literature, these findings suggest that long-term exposures to methyl bromide, in the present concentration range, are tolerated by this species. Also detailed in this report is the course of recovery of a separate group of rabbits previously given subchronic exposures to 65 ppm methyl bromide. These animals developed severe neuromuscular losses and had impaired blink reflexes and body weights. The symptoms partially subsided within 6–8 wk after removal from the exposures, suggesting that recovery from a nonfatal but seriously debilitating exposure is possible.

INTRODUCTION

Neurobehavioral impairment is a prominent feature of toxic exposures to methyl bromide. Even brief inhalation exposure to high concentrations of this fumigant can produce diverse neurological symptoms characterized by abnormal reflexes, incoordination, ataxic gait, auditory and visual disturbances, and muscular weakness (Shield et al., 1977). Memory loss, seizures, mental confusion, and depression have also been reported, but sensorimotor impairment, especially in the lower limbs, appears to dominate the clinical picture (von Oettingen, 1944). Exposures of longer duration to

Mention of product or company names does not constitute endorsement by the National Institute for Occupational Safety and Health.

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Requests for reprints should be sent to John Russo, National Institute for Occupational Safety and Health, Division of Biomedical and Behavioral Science, 4676 Columbia Parkway, Cincinnati, Ohio 45226.

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