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CASE REPORT

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A Fatal Dichlorvos Poisoning: Concentrations in Biological Specimens

ABSTRACT: A 54-year-old man was found dead with a bottle containing a brownish fluid near him. A toxicological screening was carried out in blood, urine, and stomach content. Only dichlorvos (2,2 dichlorovinyl O-O dimethylphosphate or DDVP) was found. A simple and rapid method, using DDVP-D_6 as an internal standard, was developed for the determination of DDVP by gas chromatography/mass spectrometry (GC/MS). The method was linear from 1 to 10 mg/L. Intraday and interday precisions were all <15%. DDVP concentration in cardiac blood was approximately four times higher than in peripheral blood. A high concentration was found in the heart showing a cardiac tropism of DDVP, kidney and lung concentrations being much lower. No DDVP was found in liver. DDVP stomach content was 38 g. The amount presumed ingested was 82 g, *c.* 1000 mg/kg of body. The oral LD_{50} for DDVP ranges between 20 and 1090 mg/kg in animals but is not known for humans.

KEYWORDS: forensic science, dichlorvos, tissue distribution, organophosphate pesticide

Organophosphates (OPs) are potent and effective insecticides and still represent the largest group of insecticides sold worldwide (1). The primary target for OPs is acetylcholinesterase (AChE), a B-esterase whose physiological role is that of hydrolyzing acetylcholine, a major neurotransmitter in the peripheral and central nervous system (2). Acetylcholine, released from cholinergic nerve terminals, is disposed of only through hydrolysis by AChE. Hence, inhibition of AChE by OPs causes accumulation of acetylcholine at cholinergic synapses, with an over-stimulation of muscarinic and nicotinic receptors, leading to increased sweating and salivation, profound bronchial secretion, bronchoconstriction, miosis, increased gastrointestinal motility, tremors, and muscular twitching. When death occurs, this is believed to be due to respiratory failure secondary to inhibition of respiratory centers in the brainsteam, bronchoconstriction, and paralysis of the respiratory muscles (3). Given the strong similarities of the insect and mammalian cholinergic nervous system, these compounds are responsible for the millions of poisonings and thousands of deaths occurring annually as a result of pesticide exposures, particularly in third world countries (2). Numerous efforts have been made to develop pesticides that are safe for humans based on differences in metabolism and selectivity for AChE between mammals and insects. Dichlorvos (2,2 dichlorovinyl O-O dimethylphosphate or DDVP, Fig. 1) is one of the OP insecticides that has sufficient selective toxicity to insects and thus has been widely used since 1955 (4). However, many recent publications described disorders and complications that occurred after acute DDVP intoxication in humans (5–8), but a few reported fatal intoxication (9,10). To our knowledge, fatal poisoning due to DDVP ingestion that presented DDVP quantification and tissue distribution of the compound has been reported only by two authors (11,12). In the first study (11), all DDVP concentrations were very low, probably because it was co-administrated with another OP (chlorfenvinphos), and the second study (12) was comparable to our case report, because it was also a fatal poisoning, involving ingestion of 250 mL of 75% DDVP. We report here the first French case of DDVP poisoning, with tissue distribution, assessed by a new gas chromatographic method.

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Case History

A 54-year-old Moroccan man, 174 cm in height and 80 kg in weight, was found dead in prone position on a football ground. A 250-mL bottle, originating from Morocco and found to contain 100 mL of a brownish fluid, was found near the victim. The label on the bottle mentioned "Denkavepon M50" and showed that this bottle contained 47.5% DDVP, corresponding to 550 mg/mL in xylene. Autopsy findings were diffuse congestion of the internal organs and a bleeding ulcer of the digestive tract. In the stomach, 150 mL of a volatile fluid was found. Blood (cardiac and peripheral), urine, gastric contents, and tissues (heart, lung, kidney, and liver) were collected at autopsy, frozen immediately and stored at −20°C. A systematic toxicological screening by gas chromatography/mass spectrometry (GC/MS) and liquid chromatography/diode-array detector (LC/DAD) was carried out.

Materials and Methods

Chemicals

Cyclohexan standard solutions of DDVP (10 mg/L) and DDVP-D_6 (100 mg/L) used as internal standard were purchased from Cluzeau Info Laboratory (Lyon, France). Toluene, ethyl acetate, diethyl ether, dichloromethane, hexane, isoamylic alcohol, methanol, and

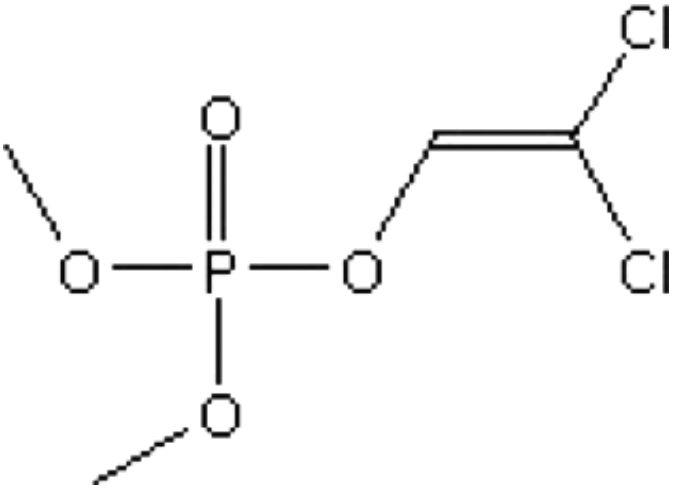

FIG. 1—*Chemical structure of DDVP.*

dimethyldichlorosilane were all HLPC grade and purchased from Prolabo (Paris, France). Sodium dihydrogenophosphate was obtained from Sigma-Aldrich (Paris, France). Phosphate buffer (pH = 5.5) was prepared by dissolving 3.45 g of sodium dihydrogenophosphate into deionized water, adjusting to pH 5.5 with 1 N NaOH.

Drug-free human plasma was supplied by the local blood bank at the Etablissement Français du Sang (Le Chesnay, France).

Sample Preparation

A stock solution of DDVP was prepared at a concentration of 10 mg/L in ethyl acetate, and DDVP-D_6 was prepared at a concentration of 50 mg/L in ethyl acetate. Both solutions were stored at room temperature in the dark. Calibration curves were prepared by spiking drug-free plasma (250 μL) with appropriate volumes of the previously mentioned DDVP working solutions to produce the calibration curve points equivalent to 1, 2, 3, 5, and 10 mg/L of DDVP. Quality control (QC) samples were prepared at 1.4 and 7.0 mg/L of DDVP by spiking two 5-mL plasma samples in bulk with appropriate volumes of DDVP working solution prepared from separate weighting. They were then aliquoted and frozen. Six aliquots of each level were thawed on each day of analysis. Silanization of the glass tubes was performed so as to minimize the adsorption of DDVP: 2 mL of toluene/dimethyldichlorosilane (95/5; v/v) were added in glass tubes. They were capped and shaken 15 min, then were rinsed by toluene twice and afterwards by methanol twice. Tissues samples (5.0 g) were homogenized in deionized water (5.0 mL) using a Polytron tissue homogenizer (Bioblock Scientific, Paris, France). Final quantification of each sample was based on an appropriate dilution in deionized water if necessary.

QC, calibration curve, blank plasma samples, and unknown samples were extracted using a liquid–liquid extraction procedure. Two hundred fifty microliter of sample, 20 μL of IS working solution, 250 μL of pH 5.5 phosphate buffer, and 4 mL of extracting solvent (dichloromethane/diethyl ether/hexane/isoamylic alcohol; 150/250/100/2.5; v/v/v/v) were added into silanized glass tubes. The samples were then shaken for 15 min and centrifuged at $3500 \times g$ for 5 min. The organic layer was decanted into another tube, where it was evaporated to complete dryness under nitrogen stream at room temperature. Samples were reconstituted with 50 μL of ethyl acetate, then vortexed for 10 sec, and 1 μL was injected into GC/MC system.

Instrumentation

The GC/MS analysis was performed using a GC 800 series gas chromatography (Thermoelectron, Les Ulis, France) and an Automass II mass spectrometer (Thermoelectron) coupled with an AS 2000 Auto sampler (Thermoelectron).

The chromatographic system was equipped with a 30 m × 0.25 mm i.d., Supelco PTE 5 5% phenyl 95% methylsiloxane column. The injector was set at 250°C and used in Splitless mode. The injector gas was Helium N55 grade (Air Liquide, Jouy-en-Josas, France) and its pressure was maintained at 70 kPa. The GC oven temperature was programmed starting at 60°C for 2 min, increased to 90°C at 40°C/min, and then from 250°C to 15°C/min. The transfer line temperature was set at 300°C. The mass spectrometer was in electron impact mode (70 eV). The MS was run first in total ion current and in selected ion monitoring mode for quantitative analysis, using m/z = 115 and 191 for DDVP-D_6 and m/z = 109 (used as quantitative ion), 145 and 185 (used as confirmation ions) for DDVP. Excalibur software (Thermoelectron) system controlled the equipment and processed data.

Validation of the Method

The recoveries were evaluated at two levels by comparing the peak areas of the extracted samples (n = 6) with those of unextracted reference standards prepared at the same concentration. For the linearity study, six calibration curves were obtained in 3 days. Linearity was tested for the 1–10 mg/L range of concentrations, employing standard calibration curves of at least five points. In addition, blank and zero plasma samples were also analyzed to confirm the absence of interferences. These two samples were not used to construct the calibration function. Quantification was performed by calculating the ratio between the peak-area of DDVP and the peak-area of IS. The mean regression curve was calculated using the least square analysis. The linearity of the regression was tested with a variance analysis (ANOVA), which separates the regression and the linearity standard deviation. The precision and accuracy of the method were carried out over 3 days. Each day, one calibration curve and six determinations of each QC level were analyzed. The values obtained were analyzed using ANOVA, which separated the intraday- and interday-assay standard deviation and, consequently, the corresponding coefficients of variation (CV). The intraday-assay CV took into account the variability of the six replicates each day for 3 days, and the interday-assay CV took into account the variability of the days of analysis. The accuracy was determined by comparing the mean calculated concentration with the spiked target concentration of the QC samples. The limit of detection (LOD) was defined as the lowest concentration of the analyte that can be detected with a signal-to-noise ratio higher than 3:1. The limit of quantification (LOQ) was defined as the lowest concentration of the compound that can be measured with both an accuracy of ±10% of the true value and a CV ≤20%.

Results and Discussion

Validation

The retention time of DDVP and IS was 7.70 and 7.73, respectively (Fig. 2). Under the chromatographic conditions used, there was no interference with the analytes by any extractable endogenous material present in plasma or in whole blood. Linearity was observed for concentrations ranging from 1 to 10 mg/L (Table 1) with a correlation coefficient >0.997. The LOD was 0.275 mg/L

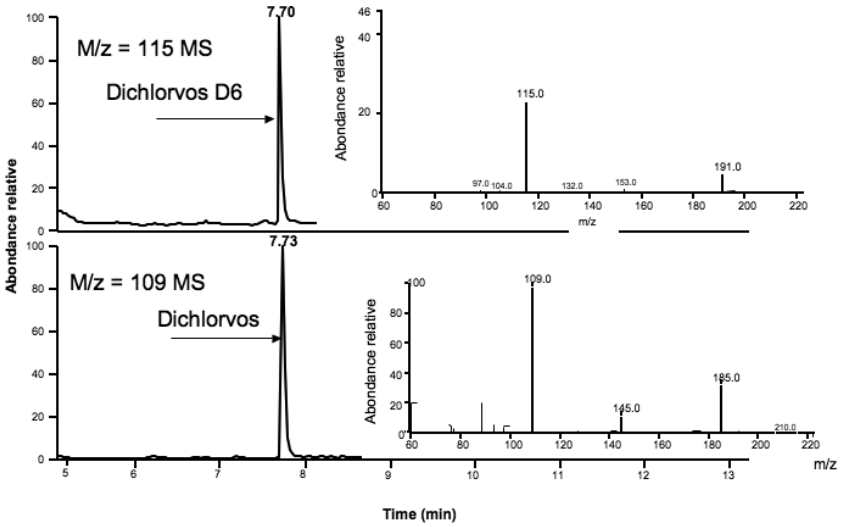

FIG. 2—*Chromatogram and mass spectrum of DDVP-D_6 (A) and DDVP (B) found in heart of the case report (1:20 dilution).*

TABLE 1—*Linearity of the method.*

	Theoretical concentrations (mg/L)				
	1	2	3	5	10
Back calculated concentrations (mean, $n = 6$)	0.99	1.99	2.89	5.19	9.94
CV (%)	11.25	11.02	10.6	3.54	0.95
Bias (%)	−0.17	−0.11	−3.77	3.95	−0.63

Six calibration curves were obtained in 3 days.

TABLE 2—*Fluid (mg/L) and tissue (mg/kg) concentrations, and stomach content of DDVP.*

	Shimizu et al. (11)	Moriya and Hashimoto (12)	This Study
Blood (not specified)	29		
Cardiac blood	–	0.043	4.4
Peripheral blood	–	ND	1.3
Brain	9.7	ND	–
Cerebrospinal fluid	–	0.027	–
Vitreous humor	–	0.067	–
Heart	815	–	1400
Lung	81	ND	2.1
Liver	20	ND	ND
Bile	–	8.99	–
Kidney	80	ND	1.0
Spleen	3340	0.542	–
Urine	4.5	ND	1.3
Stomach content (g)	300	879	38

ND, not detectable.

with a LOQ at 1 mg/L. Intraday and interday precision at 1.4 mg/L were 13.8 and 4.8, respectively, and accuracy was 100.2%. Intraday and interday precision at 7.0 mg/L were 14.8 and 9.9, respectively, and accuracy 92.7%. Relative extraction recovery was 100.2 and 111.9% for DDVP at 1.4 and 5.0 mg/L, respectively. Relative extraction recovery was 100.9% for DDVP-D_6 at 4.0 mg/L. No difference was observed between extraction recoveries in plasma or in whole blood. We used a mixture of four solvents (dichloromethane/diethyl ether/hexane/isoamylic alcohol) for extraction of DDVP because this method was also used in our laboratory for the quantification of many other OPs (parathion methyle, malathion, fenthion, parathion ethyle, chlorfenvinphos, bromophos, and coumaphos).

Case Report

Only DDVP was found by GC/MS and LC/DAD screenings in whole blood, urine, and gastric content. The method described above allowed measuring DDVP in all samples. The concentrations of DDVP found in fluid and in tissues are presented in Table 2, compared with those obtained in the two precedent studies (11,12). DDVP concentration in cardiac blood (4.4 mg/L) is approximately four times higher than in peripheral blood. A high concentration (1400 mg/kg) was found in the heart; kidney (1 mg/kg) and lung (2.1 mg/kg) concentrations were much lower. DDVP concentration of stomach content was 253 mg/mL, corresponding to 38 g of DDVP. There are probably two reasons that could explain the high cardiac concentration: a postmortem diffusion of the stomach contents or a cardiac tropism of the molecule. The phenomenon of postmortem diffusion of the stomach contents to the nearest organs such as the heart, spleen, and lung is well known. In our study, the autopsy was performed 3 days after the death. The body was found immediately after death and stored for 3 days in cold store, in prone position. The prone position helps the liquid to contaminate the organs. Furthermore, DDVP was solubilized in an organic layer (xylene), which better diffuse among the different organs. However, if the postmortem diffusion was the only cause of the high cardiac concentration, then the lung concentration should have been significantly more than that observed. So, we concluded to a high cardiac tropism of DDVP, in accordance with Shimizu et al. (11) data, showing that DDVP concentration in postmortem blood may be measured in peripheral blood and not in cardiac blood. No DDVP was found in liver, probably because of a rapid hydrolysis of the insecticide by A-esterases. The A-esterases are found in majority in the hepatic endoplasmic reticulum and can hydrolyze many OP compounds by splitting ester bonds. These enzymes are not inhibited by OP compounds probably because the phosphorylated active-site serine residue reacts rapidly with water to regenerate the free form, in contrast to its high stability in the case of the B-esterase (cholinesterase) (2). This result was in accordance with those of Shimizu et al. (11) as they found that liver concentration was very low as compared to heart or spleen concentrations. In our case

report, the ingested amount was estimated at 82 g, *c*. 1000 mg/kg of body, as 150 mL of the "Denkavepon M50" liquid was missing. The oral LD_{50} for DDVP ranges between 20 and 1090 mg/kg in animals but is not known for humans (11).

Conclusion

The simple and rapid method presented here is useful for determining DDVP concentration in biological materials. We report here the first French case of DDVP poisoning, with tissue distribution, confirming the high cardiac tropism of this molecule and its high toxicity for humans.

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