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CASE REPORT

Guillaume Hoizey,[1,2] *Ph.D.; Frédéric Canas,*[2] *M.D.; Laurent Binet,*[1] *M.S.; Matthieu L. Kaltenbach,*[3] *Ph.D.; Gérard Jeunehomme,*[2] *M.D.; Marie-Hélène Bernard,*[2] *M.D.; and Denis Lamiable,*[1,2] *Ph.D.*

Thiodicarb and Methomyl Tissue Distribution in a Fatal Multiple Compounds Poisoning

ABSTRACT: Thiodicarb is a nonsystemic carbamate insecticide whose acetylcholinesterase activity is related to its main methomyl degradation product. A 40-year-old woman was found dead in her car. Empty packages of medicines and an open bottle of Larvin® containing thiodicarb were found near her body. No signs of violence nor traumatic injuries were noticed upon autopsy, and police investigations strongly suggested a suicide. Systematic toxicological analysis performed on postmortem specimens revealed the presence of various sedatives, hypnotics, and antipsychotic drugs in blood, urine, and gastric content. Some of the compounds identified were determined at blood concentrations well above the known therapeutic concentrations: zolpidem (2.87 mg/L), bromazepam (2.39 mg/L), nordazepam (4.21 mg/L), and levopremazine (0.64 mg/L). Specific analysis of thiodicarb and of its methomyl metabolite was then performed on all fluids and tissues collected during autopsy by liquid chromatography ion trap tandem mass spectrometry (LC-MS-MS). The anticholinesterase capacity of blood, urine, and gastric content collected at autopsy was 83%, 82%, and 32%, respectively (normal value: 0%). The presence of thiodicarb in the bottle found near the body corroborates the hypothesis of an intake of that compound. Although thiodicarb was only detected in gastric content (24.3 mg/L), its methomyl metabolite was quantified in most postmortem tissues and fluids: gastric content (19.9 mg/L), peripheral blood (0.7 mg/L), urine (8.5 mg/L), bile (2.7 mg/L), liver (0.7 mg/kg), kidney (1.7 mg/kg), lung (1.5 mg/kg), brain (9.3 mg/kg), and heart (3.6 mg/kg).

KEYWORDS: forensic science, thiodicarb, methomyl, fatal poisoning, tissue distribution, liquid chromatography ion trap mass spectrometry

Thiodicarb (CAS N°59669-26-0) is a nonsystemic carbamate insecticide with a relatively narrow spectrum of activity closely related to its main methomyl degradation product (CAS N°16752-77-55). It is specific against Lepidopterous pests, controlling larvae at different stages as well as eggs (1). Its use in vineyards, under the trade name Larvin®, has been prohibited in France since 2003 (2). Thiodicarb consists of two methomyl moieties joined through their amino nitrogen by sulfur (Fig. 1). After oral ingestion in rats, it is rapidly degraded in the stomach to its methomyl derivative and some other unstable intermediates, including methomyl-methylol, methomyl-oxime, and methomyl-sulfoxide (3).

As a carbamate derivative, thiodicarb inhibits acetylcholinesterase and causes an accumulation of acetylcholine at synapses that stimulates parasympathetic postganglionic fibers and somatic motor nerve fibers. The inactivation of cholinesterase produces symptoms of intoxication that include excessive salivation, sweating, blurred vision, nausea, vomiting, seizures, and even death due to paralysis of the respiratory muscles (4). Carbamate pesticides are usually less toxic than organo phosphorous pesticides since they do not bind as strongly to acetylcholinesterase. Although fatal poisonings because of its main metabolite, methomyl, have been well described in the literature (5–7), no data has been reported, to our knowledge, with thiodicarb.

We report a case of voluntary fatal intoxication with thiodicarb by oral ingestion. Tissue concentrations of thiodicarb and of its main degradation product (methomyl) were determined by a specific liquid chromatographic method with ion trap mass spectrometry.

[1]Laboratoire de Pharmacologie et Toxicologie, CHU de Reims, Reims, France.
[2]Fédération Médico-Judiciaire, CHU de Reims, Reims, France.
[3]Laboratoire de Pharmacologie et Pharmacocinétique, UFR de Pharmacie, Reims, France.

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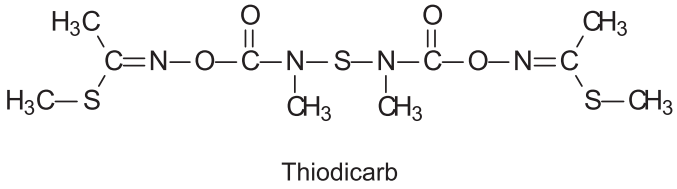
Case History

A 40-year-old woman was found dead in her car. Empty packages of various medicines and an open bottle labeled Larvin® (trade name of thiodicarb) were found near her body. An autopsy performed about 3 days after death revealed neither traumatic injuries nor signs of violence. Based on the history of the victim and in light of the evidence found on the scene, a suicide was suggested.

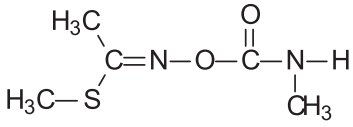
Postmortem samples submitted for toxicological analysis were femoral blood, gastric content (total volume not stated), urine, bile, vitreous humor, liver, kidney, lung, heart, and brain. The content of the bottle found near the body was also subjected to analysis. All samples were stored at –20°C until analysis.

Systematic Toxicological Analysis

Systematic toxicological analysis was first performed on femoral blood, urine, and gastric content. The presence of alcohols (ethanol, methanol, acetone, isopropanol) was explored in blood by gas chromatography (GC) with flame ionization detection. Screening for drugs of abuse in urine and for medications in blood was carried out by using an immunoassay. Determination of drugs of abuse in blood was performed using GC-mass spectrometry (GC-MS). Screening for the presence of basic and acidic drugs was performed by GC-MS, liquid chromatography with ion trap tandem mass spectrometry detection (LC-MS-MS), and liquid chromatography with photodiode-array detection (LC-DAD). Quantification of detected compounds was performed by LC-MS-MS. Owing to the



Thiodicarb



Methomyl

FIG. 1—*Chemical structures of thiodicarb and methomyl.*

conditions under which the body was found, a specific research for thiodicarb and its methomyl derivative was carried out in all samples collected during the autopsy.

Analysis of Thiodicarb and Methomyl

Thiodicarb and its main metabolite, methomyl, were specifically screened for and quantified in all samples by a LC-MS-MS method with an ion trap spectrometer.

Reagents

Thiodicarb, methomyl, and carisoprodol (internal standard) were purchased from Sigma (Saint-Quentin Fallavier, France). Acetonitrile and dichloromethane were supplied by SDS (Peypin, France). Methanol and formic acid were obtained from Merck (Darmstadt, Germany). All organic solvents and reagents were of LC grade. Purified water was prepared on a Milli-Q purification system (Millipore, Saint-Quentin en Yvelines, France).

Sample Preparation

About 100 µL or 100 mg of each body fluid or tissue homogenate (tissue:water = 1:10 w:w) was extracted with 2.0 mL of dichloromethane, after addition of 10 µL of a 200 µg/mL IS solution (carisoprodol in methanol). The mixture was vortex mixed for 1 min, then centrifuged at 3000×*g* for 5 min. The organic layer was transferred into conical glass tubes and evaporated to dryness under a nitrogen stream at 40°C. The residue was then dissolved in 200 µL of 0.1% formic acid:acetonitrile (50:50 v:v), and 10 µL was injected into the LC column.

Liquid Chromatography-mass Spectrometry

The LC-MS-MS system consisted of a Thermofinnigan Surveyor® LC system (Les Ulis, France) equipped with an autosampler. Compounds were screened for, identified, and quantified in biological samples using a Thermofinnigan LCQ Advantage® trap ion mass spectrometer with a Thermofinnigan Xcalibur® data system. Chromatographic separations were carried out by using a 5 µm particle size Hypurity C18 column (150 mm × 2.1 mm i.d., ThermoHypersil-Keystone, Les Ulis, France) whose temperature was maintained at 30°C. Samples were eluted with a mobile phase consisting of acetonitrile: 0.1% formic acid in purified water (50:50, v:v) delivered at a flow-rate of 0.3 mL/min. The entire flow was directed into the source without splitting. During use, the mobile phase was degassed by an integrated Surveyor® series de-gasser. In order to optimize the MS-MS parameters and to expand the spectra library, infusion experiments with thiodicarb and methomyl were carried out with a syringe connected to a pump set at a flow-rate of 5 µL/min.

Electrospray ionization (ESI) in the positive-ion mode was used as the ionization technique. The spray needle was set at a potential of 4 kV. The heated capillary was set at 200°C, and the stainless-steel capillary held at a potential of 10 V. Nitrogen was used both as drying and nebulizing gas. The sheath gas flow rate of nitrogen was set at 40 (arbitrary units). The tube lens offset was set at 40 V, and the electron multiplier voltage set at 400 V peak-to-peak. Ultra-pure helium (99.995%) was used in the trap as damping and collision gas.

The detection of thiodicarb, methomyl, and carisoprodol was performed in full MS-MS scan mode (*m/z* 100–400). Three alternating scan events, generating fragment ions of the molecular ion through collision induced dissociation (CID), were carried out at *m/z* 355, *m/z* 163, and *m/z* 261 corresponding to the protonated molecular ions $[M + H]^+$ of thiodicarb, methomyl, and carisoprodol (IS), respectively. Full scan MS-MS spectra were produced by CID of each molecular ion using a normalized collision energy of 50%. Table 1 presents the MS-MS spectra data and retention times of the compounds of interest. A representative chromatogram of extract of thiodicarb and methomyl detected in the case's gastric content is presented in Fig. 2.

The reference MS-MS spectra of the compounds of interest were previously collected individually using direct injection *via* the integrated syringe pump. Those spectra were obtained by using a normalized collision energy of 50%, and were included in a custom full MS-MS library. Positive peaks were identified by searching and comparing the underlying ESI mass spectra with the reference spectra of our home-made MS-MS library.

Quantitation was also performed in the full scan MS-MS mode. Once full mass spectra of the product ions were generated, post-acquisition data processing was designed to select particular ions for quantitation (usually, fragment ions with the greatest intensity). Peak area ratios of the target ions of each compound *versus* that of the IS were compared with calibration curves prepared under the same conditions.

Being a method to be used in a single case analysis, the validation was reduced to a limited number of experiments in blood, as recently proposed by F.T. Peters et al. (8). Calibration curves were linear up to 50 mg/L for thiodicarb and 20 mg/L for methomyl in whole blood (five concentration levels). The limit of quantitation (LOQ, based on a signal-to-noise ratio equal to or > 10) for thiodicarb and methomyl in blood were 0.5 µg/mL and 0.1 µg/mL, respectively. Precision (within-run evaluation) of the method, determined at two levels (1 and 5 µg/mL for both compounds), yielded

TABLE 1—*Retention times and MS-MS spectra for thiodicarb, methomyl, and carisoprodol (internal standard).*

Compound	Rt (min)	Parent Ion (*m/z*)	Daughter Ions (*m/z*)*
Thiodicarb	2.31	355 $(M + H)^+$	163 (100), 209 (26), 193 (17)
Methomyl	1.76	163 $(M + H)^+$	122 (100), 88 (63)
Carisoprodol	2.66	261 $(M + H)^+$	176 (100), 158 (22), 200 (11)

Note: Ions underlined were used for quantitation.
*Values in brackets are the relative intensities

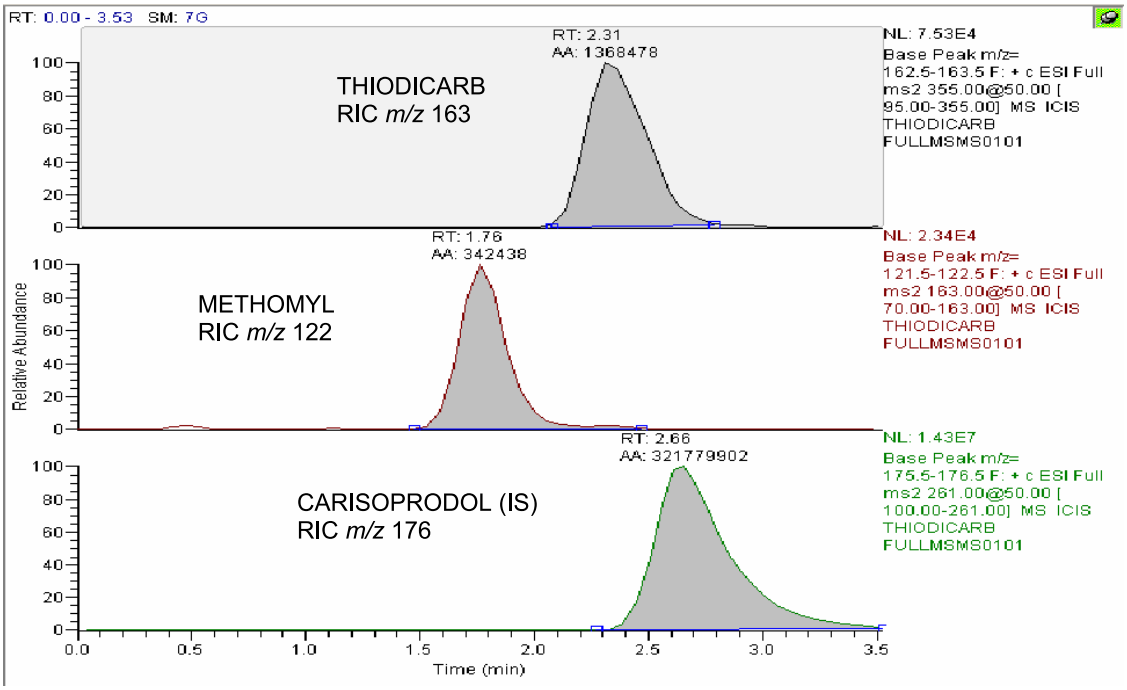


FIG. 2—*Reconstructed LC-MS-MS ion chromatograms (RIC) of extract of thiodicarb (24.3 mg/L) and methomyl (19.9 mg/L) detected in gastric content in a fatal case involving thiodicarb ingestion. Carisoprodol was used as internal standard.*

relative standard deviations lower than 15% (six replicates per level).

Determination of the Anticholinesterase Capacity

Anticholinesterase capacity of blood, urine, and gastric content was determined by using a modification of the method of Mahieu et al. (9). This parameter provides a rapid answer on the presence or absence of a direct cholinesterase inhibitor in biological samples. Briefly, cholinesterase activity (P) was determined in all samples by using the Flex® Pseudocholinesterase assay (Dade Behring, Paris, France) and in a blank control serum (T). Equal volumes (100 μL) of the blank control serum and of either blood, urine, or gastric content collected at autopsy were then incubated at 37°C for 15 min, and the cholinesterase activity determined in each mixture (M). The anticholinesterase capacity of postmortem samples, expressed as a percentage, was then computed from the following equation:

$$100 \times \left[1 - \frac{2 \times (M)}{(T) + (P)}\right]$$

As such, a value of 0% is indicative of the lack of cholinesterase-inhibiting compounds in the sample tested.

Results and Discussion

No ethyl alcohol and no drug of abuse was found in any of the samples investigated. Systematic toxicological analysis showed the presence of various sedatives, hypnotics, and antipsychotic drugs in all samples. As shown in Table 2, peripheral blood concentrations of some of the drugs identified were well above known therapeutic concentrations (10). It should be noted that the systematic toxicological analysis did not permit the detection of both thiodicarb and methomyl in any of the samples. This is mainly due to the fact that these compounds were not present in our spectral LC-MS-MS and LC-DAD libraries at the time at which the analysis was performed. Additionally, the well-known poor stability of carbamates under high temperatures as needed for GC analysis, may render difficult their identification by GC-MS screening (5,6). The presence of an open bottle labeled Larvin® on the suicide scene suggested the possibility of an ingestion of thiodicarb by the victim. The analysis of the bottle content confirmed the presence of thiodicarb.

TABLE 2—*Blood concentrations of compounds identified by systematic toxicological analysis conducted on postmortem samples (blood, urine, gastric content) collected during autopsy of a 40-year-old woman.*

Compound	Blood Concentration (mg/L)
Zolpidem	2.87
Lormetazepam	0.057
Nordazepam	4.21
Tetrazepam	0.35
Bromazepam	2.39
Levopromazine	0.64

The anticholinesterase capacity of blood, urine, and gastric content was 83%, 82%, and 32%, respectively (normal capacity: 0%). These values clearly demonstrate the presence of a direct cholinesterase inhibitor in those samples.

Results of the LC-MS-MS analysis of thiodicarb and methomyl in postmortem samples are presented in Table 3. The lack of knowledge of the exact volume of gastric content collected during autopsy did not allow us to estimate the amount of thiodicarb and methomyl present in the stomach at death.

Unstable under acidic conditions, thiodicarb is rapidly hydrolyzed in its methomyl derivatives in the stomach. This is in agreement with our results showing the presence of thiodicarb only in gastric content, whereas none was detected in any other postmortem tissues or fluids. However, the acid environment of the stomach cannot be the only explanation for the absence of thiodicarb in

TABLE 3—*Thiodicarb and methomyl concentrations in body fluids and tissues of a 40-year-old woman who died after taking thiodicarb (Larvin®).*

Fluids or Tissues	Thiodicarb (mg/L)	Methomyl (mg/L) ou (mg/kg)
Gastric content	24.3	19.9
Peripheral blood	<LOQ	0.7
Urine	<LOQ	8.5
Bile	<LOQ	2.7
Vitreous humor	<LOQ	<LOQ
Liver	< LOQ	0.7
Kidney	<LOQ	1.7
Lung	<LOQ	1.5
Brain	<LOQ	9.3
Heart	<LOQ	3.6

other fluids and tissues, since rapid metabolism of thiodicarb in blood and organ tissues may account for much of the lack of detection of thiodicarb in these specimens. In contrast, various concentrations of methomyl were detected in all postmortem samples, except in vitreous humor. Those concentrations were in the range of those reported in similar fatal cases, although highly variable between each case. For instance, blood and liver methomyl concentrations following methomyl ingestion in several published fatal case-reports varied between 0.003 and 63.5 mg/L, and between "not detectable" and 1.2 mg/kg, respectively (7,11,12).

Concentrations of methomyl in most tissues and fluids, particularly brain and heart, were higher than in peripheral blood. These findings, indicative of an extensive ante-mortem distribution of methomyl, are in agreement with the high lipid solubility of methomyl (13). In contrast to the cases reported by Moriya et al., methomyl was not detected in vitreous humor, although this biological matrix was described as a specimen of choice for detecting carbamates postmortem (7,12).

The interpretation of our results remains difficult, mainly owing to (1) the limited number of articles published in the literature, (2) the lack of information concerning the delay between thiodicarb ingestion and death, (3) the instability of sulfur containing compounds, such as methomyl, even in frozen postmortem biological samples (13,14), and (4) the occurrence of a potential postmortem redistribution, and biotransformation of methomyl after death because of the activity of carboxylesterases in blood and tissues (7).

Fatal poisoning involving the self-ingestion of thiodicarb and/or methomyl are not well documented in the literature, especially in France, where no case of self-poisoning with thiodicarb has been reported to date. In this case, police investigations indicated that death was likely because of a suicide. Although toxicological analysis does not permit to conclude that death was solely because of the intake of Larvin® (thiodicarb), the important inhibition of cholinesterase activity related to the presence of methomyl may have caused a fatal respiratory paralysis, which could have been potentiated by the presence, at toxic blood concentrations, of various respiratory depressant drugs.

The evaluation of thiodicarb and methomyl tissue distribution was helpful to investigate their fate in body fluids and tissues in postmortem samples. Our results showed that, after oral ingestion, thiodicarb is not detected in any of the tissues or fluids analyzed. It also confirmed the important distribution of methomyl throughout the body.

Finally, this case highlights the fact that deliberate ingestion of stored agricultural pesticides that are no longer being produced can still occur.

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Additional information and reprint requests:
Guillaume Hoizey, Pharm.D., Ph.D.
Laboratoire de Pharmacologie et Toxicologie
Hôpital Maison Blanche
CHU de Reims
45, rue Cognacq-Jay
51092 Reims Cedex
France
E-mail: ghoizey@chu-reims.fr