Toxicological Analysis in a Case of Endosulfan Suicide

J. Demeter[1], A. Heyndrickx[1], J. Timperman[2], M. Lefevere[1], and J. De Beer[1]

[1]*Department of Toxicology*
State University of Ghent
B-9000 Ghent, Belgium
Hospitaalstraat 13

[2]*Department of Legal Medicine*
State University of Ghent, Belgium
Senior Lecturer Free University of Brussels, Belgium

ABSTRACT

Stomach, small intestine contents, blood, liver, kidney and urine of a 28-years old man, were analyzed for residues of Endosulfan (6, 7, 8, 9, 10, 10-hexachloro-1, 5, 5a, 6, 9, 9a-hexahydro-6, 9-methano-2, 4, 3-benzo(e)dioxathiepin 3-oxide).
The analysis results showed the presence of high concentrations of the two endosulfan isomers in all samples. Since also alcohol was present in all the tissues analyzed, it was concluded that the victim died of a combined endosulfan-alcohol poisoning.
No other drugs were found.

CASE HISTORY

On January 12th 1975, at 4 o'clock in the morning, an ambulance received an urgent call with request to bring a man in desperate condition to the nearest hospital. On arrival there he was already dead. The victim, a bachelor of 28 years, had, the day before, sprinkled rose bushes with Thiodan^{R} powder. That evening he enjoyed his supper as usual and, afterwards, dressed to go out. He came back drunk at 3 o'clock in the morning. After a few words with his mother, he seemed to go to sleep. A short time later his mother was again awakened by some noise. She went to the bedroom and found her son lying on the floor, severely ill, trying to vomit.

POST-MORTEM EXAMINATION

External examination :

The body was that of a powerfully built young man weighing 72 kg and measuring 168 cm. The livores were dark red-purple. Some recent, insignificant excoriations and a few scars were visible.

Internal examination :

Head : No particularities were to be reported except a marked congestion of the meningeal vessels.

Bulletin of Environmental Contamination & Toxicology,
Vol. 18, No. 1 © 1977 by Springer-Verlag New York Inc.

Chest : The right lung weighed 855 g; the left 755 g. They were congested and considerably edematous. Most small air passages were obstructed by vomited matter. The heart was slightly dilated and weighed 470 g.

Abdomen : The liver was dark coloured, congestive and weighed 1 840 g. The stomach only contained brownish liquid. he mucosa, near the cardia, showed marked congestion. The suprarenals and the pancreas presented no abnormalities. The enlarged spleen weighed 140 g and was muddy. The kidneys weighed 180 g and 170 g, and were congested.

The skeleton showed no abnormalities. The blood was everywhere abundant, extremely fluid and cyanotic.

Microscopic examination :

The histological findings were rather poor. There was slight perivascular fibrosis in the myocardium. Discrete proliferation of the fibrous tissue and infiltration thereof by lymphocytes was visible in the liver. The kidney showed a few subcapsular scars. The presence of hemorraghic edema in the lungs and of alimentary detritus in the small airways was confirmed. The brain parenchyma only showed congestion.

The autopsy findings strongly suggesting an asphyxial death due to poisoning, stomach and small intestine contents, blood, a fragment of liver, a kidney and the urine,together with a residue of the manipulated powder(Thiodan^{R}), were taken for toxicological analysis.

MATERIALS AND METHODS

The endosulfan standards of analytical grade were supplied by Riedel-De Haën AG, Seelze-Hannover.
All reagents used were of analytical grade. Benzene and methylene chloride were purified as prescribed by VOGEL (1967). n-Hexane was refluxed overnight with potassium hydroxide pellets (4 g/L) and distilled.

Isolation of endosulfan from the commercial formulation

The commercial formulation (500 mg), found near the victim was mixed with anhydrous sodium sulphate (1 g) and activated charcoal (1 g) in a mortar.
The powder was transferred into a chromatographic column and eluted with 70 ml 20 percent methylene chloride in n-hexane (v/v). The eluate was concentrated in a Kuderna-Danish evaporator and the remaining extract further evaporated under a mild N_2-stream at 30° C in a warmwaterbath. Prior to gaschromatography, the residue is dissolved in an appropriate volume of n-hexane.

Isolation of endosulfan out of the post-mortem sample

1. Stomach content (5 g), small intestine content (30 g), blood (20 g), liver (10 g), kidney (10 g) and urine (5 g) are mixed thoroughly with acid washed sea-sand (10 g) and anhydrous sodium sulphate q.s. until a homogenous dry powder is obtained. This mixture is extracted with 100 ml acetonitrile in an erlenmeyer flask. The organic layer is decanted and filtered. The remaining extraction residue is washed with 10 ml portions of acetonitrile until the volume of the combined organic layers is 100 ml.

2. The combined acetonitrile portions are washed once with 100 ml n-hexane, saturated with acetonitrile. To prevent excessive losses, the n-hexane layer is washed twice with 50 ml of acetonitrile, saturated with n-hexane. All the acetonitrile layers are combined in a rotavapor flask and evaporated to approximatively 20 ml in a rotavapor apparatus.

3. The concentrated extract is poured into a separation funnel, containing 300 ml of a 5 percent sodium sulphate (w/v) solution in water and 20 ml of benzene.
After thorough mixing for at least 2 minutes, the layers are allowed to separate. The benzene layer is isolated and evaporated to 5 ml in a graduated test tube under a gentle N_2-stream.

4. 2,5 ml are cleaned up by a 7 mm i.d. x 250 mm chromatographic column filled with 6,5 g (± 15 cm) 5 percent water desactivated neutral alumina, as described by HOLDEN and MARSDEN (1969). Prescriptions of CLAEYS and INMAN (1974) in preparation and application of adsorbents are taken into account. Elution was performed by 70 ml 20 percent methylene chloride in n-hexane.

5. To perform recovery experiments aldrine was chosen as internal standard. Aldrine is added to the eluate and this is concentrated (see isolation procedure for the commercial formulation). Appropriate dilutions are made with n-hexane.

Detection

A Hewlett-Packard model 5750 G gaschromatograph equipped with a tritium electron capture detector and a 6' x 4 mm i.d. column containing 3,8 percent UCCW-982 on Chromosorb W HP, 80-100 mesh is used.

Col. temp. : 200° C, detector temp. : 210°C, injection port temp. : 210° C, injection port temp. : 240° C and a Helium flow rate of 56 ml/min. The Purge gas used was a 10 percent methane-90 percent argon mixture with a flow rate of 43,5 ml/min. Pulse internal was 150. Results were confirmed on a mixed column, containing 1,5 percent OV-17 and 2 percent QF-1 on Chromosorb W HP, 80-100 mesh.

Recovery experiments

The extraction procedure of α- and β-endosulfan in biological material consists of five steps, according to the given isolation procedure.

Results, as given in TABLE I and covering the whole extraction procedure, suggest that different recoveries for α-and β-endosulfan are mainly obtained as a results of different partition coefficients in the hexane-acetonitrile binairy solvent system : the p-values are 0.39 and 0.13 for α- and β-endosulfan, respectively (BEROZA, M. and BOWMAN, M.C. 1965)

TABLE I

Recovery of added endosulfan over the whole extraction procedure

Fluid or biolog. material spiked	Isomer	Number of experiments	Added endosulfan ppm	Found endosulfan ppm (Mean)[1]	Recovery percent (95 percent confidence int.)
KIDNEY LIVER	α	6	0,150	0,120-0,133 (0,1255)	83,6 ± 3,4
	β	6	0,200	0,17-0,21 (0,19)	95,8 ± 7,7

[1]No values are to be rejected at the 95 percent confidence level of significance. Values are rounded off in accordance with a normalized procedure (NEN 1047)

The higher recovery of β-endosulfan is also due to difference in the physical properties between aldrine (melting point 104° C) as internal standard and β-endosulfan (melting point 208° C). Aldrine is proved to be a more suitable internal standard for α-endosulfan (melting point 108° C) determinations.

RESULTS AND DISCUSSION

Table II contains the results found in the various tissues of the victim. The ingested commercial formulation consists of 12,4 percent α-endosulfan and 8,1 percent β-endosulfan. The α/β ratio is 1,53. Results are showing that the α/β ratio is the same in the commercial formulation and in stomach content.
An increase of the α/β ratio occurred in all other samples. The method used offers the possibility to extract endosulfan with a high yield and an acceptable reproducibility.

TABLE II

Distribution and concentrations of endosulfan in different tissues of the victim

BIOLOGICAL SAMPLE	α-ENDOSULFAN ppm	β-ENDOSULFAN ppm	α/β RATIO[1]	ALCOHOL g/l
Stomach content	2 610	1 900	1,59	-
Small intest. content	190	99	2,19	-
Blood	0,06	0,015	4,50	1,81
Liver	12,4	5,2	2,71	-
Kidney	2,48	1,8	1,62	-
Urine	1,78	0,87	2,04	2,47
COMMERCIAL FORM.	12,4 percent	8,1 percent	1,53	

[1]Values adapted to the recovery experiments results

Since no other fatal intoxications by endosulfan are reported in the international literature, only the results of TABLE II can be taken into consideration.
It is obvious that the alcohol concentrations can in no way be considered lethal.
On the other hand it would be rash to make endosulfan alone responsible for the fatal outcome, considering the values of the figures at hand. We feel entitled to draw the conclusion that the man, when back home, while drunken, ingested a large quantity of Thiodan^{R} and died forthwith from the combined and possible synergic effect of both substances. We can safely exclude that the poisoning occurred accidentally while he was taking care of his roses.

REFERENCES

BEROZA, M. and BOWMAN, M.C. : J. Ass. Off. Anal. Chem., 48, 358 (1965)

CLAEYS, R.R. and INMAN, R.D. : J. Ass. Anal. Chem., 57, 399 (1974)

HOLDEN, A.V. and MARSDEN, K. : J. Chromatogr., 44, 481 (1969)

VOGEL, A.I. : Practical Organic Chemistry. 3 ed. London : Longmans 1967