GLC Determination of Crufomate (Ruelene) in Bovine Blood and the Use of UV Irradiation as a Confirmatory Test

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Crufomate (Ruelene, 4-tert butyl-2-chlorophenyl methyl methylphosphoramidate) has been in use as a systemic insecticide for over 10 years. During this time fly brain cholinesterase bioassay has been the established method of analysis. In the past few years, GLC data and methods for crufomate have been reported (1,2,3), and most recently by Rice and Dishburger (4) for residues in animal tissue and blood at the 0.02 ppm level.

Independantly, another method with a similar sensitivity had been developed in these laboratories whilst examining bovine blood for crufomate and possible toxic metabolites. This paper describes the procedure, the precision of the crufomate determinations using crude blood extracts for analysis, compares the results with the bioassay and describes a simple confirmatory test for crufomate.

Experimental

Materials - Heparinized venous blood was taken from cattle treated with crufomate 25E formulation as "pour-on" along the back at a level 0.96×10^{-2} oz/lb for the low dose. Samples were collected at the Research Station, Lethbridge, Alberta where the bioassay was performed (5). The samples were packed in dry CO_2 and shipped to these laboratories, where they were kept at -4° until extracted and analyzed by GLC.

GLC Conditions - A Pye gas chromatograph model 154, equipped with a cesium bromide thermionic detector was employed. Glass columns, 0.9m x 4 mm I.D. were packed with 100/120 Gas Chrom Q coated with 3% OV-17 (Applied Science Labs, State College, Pa.). The columns were conditioned at 340° for 4 hr, and 250° for 24 hr with a nitrogen flow of 30 ml/min. The detector response was adjusted for 1/2 f.s.d. to 1 ng crufomate by varying the hydrogen flow, this required an attenuator setting of 10×10^2. With a column and detector temperature of 220° and a column flow of 30 ml/min nitrogen, the retention time of crufomate was 4.18 min (1.19 relative to parathion). The lowest level of detection was 0.25 ng and the response was linear as far as it was tested to 50 ng.

Contribution No. 714. Chemistry and Biology Research Institute

Bulletin of Environmental Contamination & Toxicology,
Vol. 7, No. 4, 1972, © by Springer-Verlag New York Inc.

Extraction and GLC Analysis - A 5 ml blood sample was extracted with 3 x 3 ml aliquots of hexane or chloroform, the extracts had to be centrifuged in order to break-up the emulsion formed. The extracts combined and taken to dryness at room temperature with a stream of filtered air. The residue was made up to 0.5 ml with hexane and 2 µl injected without further clean-up. Each blood sample was bracketed by 2 µl crufomate standard (1 ng), and the amount of crufomate in blood determined using the averaged peak height of the neighbouring standards. Determinations were carried out in triplicate.

Irradiation - Crufomate (50 mg) in hexane (250 ml) was irradiated using a Penray U.V. lamp. (Intensity, Av. 2537°A, 2400 mw at 1"). After 30 min all the crufomate had disappeared leaving a single product with a retention time 2.8 min, (0.67 relative to crufomate), yield 78%. The same lamp was used to irradiate residue samples, which were dissolved in 5 ml hexane.

Mass Spectrometer - A C.E.C. model 490 was used with a 70 e.v. source. A 2 µg sample was chromatographed with the same GLC apparatus and conditions as above, and introduced into the mass spectrometer via a beam jet separator.

Results and Discussions

Methods for the extraction of organochlorine and organophosphorus insecticides from blood by solvents such as hexane and acetone/ether, followed by direct GLC analysis of the crude extracts are recorded in literature (6,7). The efficiency of various solvents in recovering crufomate from bovine blood fortified with standard amounts of crufomate in acetone was studied. The results are shown in the following table.

TABLE I

Recovery of crufomate from fortified bovine blood [1]

Solvent	Recovery % [2] 0.05 ppm	0.025 ppm
Hexane	74	67
Benzene	116	-
Ether	96	-
Chloroform	89	78

[1] 5 ml blood fortified with the crufomate in 25 µl acetone, shaken and allowed to stand for 15 mins.

[2] average of triplicate experiments

The best chromatographic backgrounds were obtained with chloroform and hexane, and both these solvents were used in this work.

The use of crude extracts of blood for glc analysis of crufomate is justified by the results shown in Table 2. This table shows the precision of the crufomate determination in standards, crude blood extracts and standards used in their evaluation. The precision is homogenious according to Snedicor's criteria (8), [$F = 8.6/6.2 = 1.59$, $F_{0.05} = (55/36) = 1.8$].

TABLE 2

Precision of crufomate determinations in hexane and crude extracts of blood

SAMPLE	AMOUNT (ng)	NO. OF DETERMINATIONS	REL. STD. DEV. (%)
Standard in HEXANE	5	10	4.23
	2	37	6.83
	1	9	9.26
Standard used in BLOOD ANALYSIS	2	56	8.63
Crude Extract of BLOOD	1.28	14	6.15

In addition the reproducibility of the analysis of a crude extract of blood is also comparable with that of crufomate standards in hexane. During the time taken for the analysis of blood extracts and crufomate standards, the column and detector performance did not appear to have been influenced by the use of crude samples.

Once crufomate had been extracted from blood, it was comparatively stable at ambient temperatures. Analysis of the hexane extracts of cattle blood were repeated 3 months later and gave identical results within the limits of experimental error. However, storage of the blood samples for long periods of time at 10° prior to extraction, resulted in considerable loss of crufomate.

A comparison of the GLC and bioassay results for the analysis of crufomate in the blood of a treated animal is shown in fig. 1. The two methods are in good agreement.

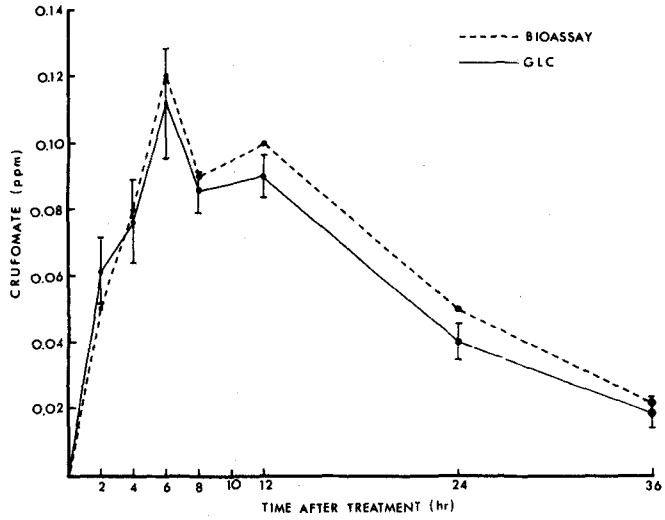

Fig. 1. Comparison of the GLC and bioassay analysis of crufomate in bovine blood

The blood of cattle treated at a higher dose level was extracted by both chloroform and hexane. The results (fig. 2), indicate that chloroform is the better solvent, although the difference in the amount extracted is not as great as expected from the work with fortified samples.

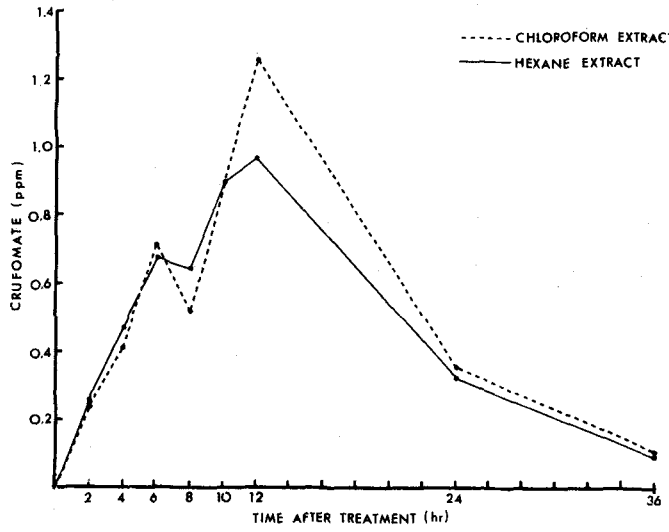

Fig. 2. Comparison of the hexane and chloroform extracts of bovine blood containing crufomate

Some organophosphorus insecticides possessing $-N(CH_3)_2$ and $-NH(CH_3)$ groups are degraded by oxidases to yield toxic des methyl derivatives (9). No other phosphorus containing compound was detected in the above work, although des N-methyl crufomate is readily detectable under the GLC condition used. Thus crufomate is the only cholinesterase inhibitor present in bovine blood. This agrees with the work of Bauriedal and Swark (10), who found only acidic degradation products in fortified sheeps blood using paper chromatography.

Irradiation of crufomate in hexane by a low intensity UV source resulted in its convertion to a compound with a retention time of 2.8 min. Analysis of this compound by GLC/ms technique showed a parent peak at 257 m/e together with peaks at 243, 148, 135, 107 and 91 m/e with a relative abundancy of 100, 62, 60, 19 and 17%. Comparison with the fragmentation pattern of crufomate, which showed a parent peak at 291 m/e together with a P + 2 peak at 293 m/e, suggested the loss of the chloro group. Such reactions are well known on the irradiation of herbicides which contain an aromatic chloro moiety (11). The ^1H nmr of the irradiated compound agrees with this hypothesis, since the aromatic protons exhibit a simple AB spectrum (J = 8.5 HZ) and the tertbutyl methyl protons (τ = 8.61) are down field relative to those of crufomate. The structure of 4-tert butyl-phenyl methyl methylphosphoramidate is suggested for this product.

This reaction was also carried out at the residue level. Irradiation of a crude blood extract (0.12 ppm) in hexane for 30 min at ambient temperature gave the same product. The conversion at this level was of the order of 67%, based on the relative peak area.

Summarizing, extraction of bovine blood with chloroform and chromatography of the crude extracts gave a good recovery of crufomate at the 0.025 ppm level. The method compares well with the bioassay method. No neutral metabolites were found in the hexane or chloroform extracts of blood. UV irradiation of crufomate results in the formation of a compound thought to be the des chloro derivative, this reaction provides a facile confirmatory test at the residue level.

Acknowledgements

We wish to thank Dr. H. Dishberger, Dow Chemical Co., Midland, for analytical samples of crufomate and its des methyl derivative, 4-tert butyl-2-chlorophenyl methyl phosphoramidate. Also we wish to thank Mrs. M. Wilson for the GLC determinations together with all the calculations, and Mr. S. Skinner for the mass and nmr spectra.

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