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DETERMINATION OF 2,3,7,8-TETRACHLORODIBENZO-1,4-DIOXIN AT PARTS PER BILLION* LEVELS IN TECHNICAL-GRADE 2,4,5-TRICHLOROPHENOXYACETIC ACID, IN 2,4,5-T ALKYL ESTER AND 2,4,5-T AMINE SALT HERBICIDE FORMULATIONS BY QUADRUPOLE MASS FRAGMENTOGRAPHY

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SUMMARY

A fast, highly selective gas chromatographic method was developed for the determination of 2,3,7,8-tetrachlorodibenzo-1,4-dioxin (TCDD) in technical 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and also in 2,4,5-T alkyl ester and amine salt herbicide formulations using quadrupole mass fragmentography. The method is faster, more sensitive and has a higher specificity than previously reported methods using conventional gas chromatography. It requires minimal or no sample clean-up. The content of TCDD found in batches of 2,4,5-T produced from 1967 to 1973 by one manufacturer showed a sharp decrease after 1970 from 500 to 50 ppb.*

INTRODUCTION

Polychlorinated dibenzo-1,4-dioxins, especially 2,3,7,8-tetrachlorodibenzo-1,4-dioxin (TCDD), are extremely toxic[1,2], teratogenic[3] and mutagenic[4] compounds. Owing to their stability in biological systems[5,6] and their potential for accumulation through food chains[7], they present a threat to man, animals and the environment. In order to prevent environmental contamination with TCDD and thus to prevent irreparable biological damage, the further use of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and other chemically related pesticides possibly contaminated with TCDD must therefore depend on the purity of the active ingredient. The Swiss Federal Research Station at Waedenswil, as the authority for the licencing of agricultural pesticides in Switzerland, established a specification for a maximum content of 50 ppb* of TCDD in 2,4,5-T. Similar specifications for TCDD in 2,4,5-T have been adopted in other countries. There has been a considerable international effort in the development of methods[1,6,8–11] to enable manufacturers and government laboratories to control the purity of these products. As a contribution, this paper describes a simple method that permits the rapid and accurate determination of

* Throughout this article the American (10^9) billion is meant.

TCDD at the required parts per billion level in technical-grade 2,4,5-T and its formulations. Mass spectrometry is widely used for pesticide analysis[12]. But mass fragmentography so far has been used mainly for the analysis of drugs and their metabolites in biological systems[13–15]. We assume that this technique will also gain more and more acceptance in the fields of pesticide residue analysis and formulation control.

EXPERIMENTAL

Reagents

Methanol, diethyl ether and ethyl acetate were obtained in puriss quality and light petroleum (b.p. 40–65 °) and lithium hydroxide in purum quality from Fluka (Buchs, Switzerland). Aluminium oxide (basic, cationotropic) from Woelm (Eschwege, G.F.R.) was used as received.

Reference compound

2,3,7,8-Tetrachlorodibenzo-1,4-dioxin (TCDD) was obtained from Stickstoffwerke Linz (Linz, Austria) and its purity was checked by gas chromatographic (GC), mass spectrometric (MS) and micro-elemental analysis. It was found to be suitable for use as a standard without further purification. Standard solutions of TCDD in ethyl acetate were prepared at concentrations of 1 and 10 μg/ml. The safety precautions to be taken when handling this highly toxic compound have been stressed elsewhere[11].

Instrumentation

A Finnigan Model 1015D quadrupole gas chromatograph–mass spectrometer was used to obtain conventional mass spectra on the reference compound. A GC column was interfaced with the mass spectrometer via a venting system and a glass jet separator. The glass column (1.5 m×2 mm I.D.) was packed with 3% silicone OV-225 on Chromosorb W AW-DMCS. The column had been previously treated with silylation reagent. The helium carrier gas flow-rate was adjusted to 25 ml/min. The injection port temperature was 250 °, the column temperature 230 ° and the separator temperature 250 °. Mass spectra were recorded at 70 eV. The ion energy used was approximately 10 V and the electron multiplier voltage 3 kV. A scan time of 2 sec was found to be suitable for an *m/e* range of 35–550.

For mass fragmentography, the instrument was operated in a similar fashion. With the instrument set at unit resolution, the ion intensities at *m/e* 320 for TCDD were monitored (single ion detection). This adjustment was made using the precision mass meter of the instrument. The signals were recorded on a regular 10-mV recorder using an additional 0.16-Hz filter.

A second, similarly treated glass column with an auxiliary T-port at approximately one third of the column length from the inlet was used for back-flushing possible late-eluting, interfering substances. This column was packed in the first portion (50 cm) with 3% Carbowax 20M on Chromosorb W AW-DMCS, and in the second portion (100 cm) with 3% silicone OV-225. The column was installed in the gas chromatograph with helium carrier gas lines connected to both the inlet port and the auxiliary port. Additionally, venting lines were mounted for the inlet port and

auxiliary port. The carrier gas and venting lines could be individually switched on and off by toggle valves outside the chromatograph. Operation of this column at 240° was done with constant pressure (2.0 atm) rather than constant flow-rate in order to achieve efficient back-flushing. The total retention time for TCDD on both this composite column and on the first conventional column was between 5 and 6 min.

Sample preparation

A sample preparation based on the work of Edmunds *et al.*[11] was used. Twenty grams of sample were dissolved or suspended in 150 ml methanol and 25 ml of 5 *N* lithium hydroxide solution were added. The mixture was refluxed for 20 min and then cooled to room temperature, and 500 ml of water and 200 ml of light petroleum were added and the mixture was vigorously shaken. After separation of the phases, the organic phase was dried over anhydrous sodium sulphate. A portion of about 100 ml was concentrated under vacuum on a rotary evaporator (maximum temperature 50°) to about 10 ml. An aliquot was usually taken because some formulations formed emulsions that were difficult to separate.

The concentrate was transferred to an alumina column (45 ml of basic aluminium oxide in a 50 cm×20 mm I.D. column). The column was eluted with 100 ml of light petroleum, 50 ml of 5% diethyl ether and then by 100 ml of 25% diethyl ether in light petroleum. These eluates were discarded. TCDD was eluted with 150 ml of diethyl ether and the solution was concentrated to approximately 10 ml. After transfer to a suitable receiver, it was further concentrated to about 0.2 ml with a gentle stream of nitrogen. Care was taken never to allow the sample to be evaporated completely to dryness. The samples, made up to 1.0 ml with ethyl acetate, were now ready for GC–MS analysis.

A shortened procedure was used for technical 2,4,5-T or 2,4,5-trichlorophenol by omitting the refluxing and the clean-up steps. To a 20-g sample, dissolved in 150 ml of methanol, 500 ml of water and 25 ml of 5 N lithium hydroxide solution were added. After addition of 200 ml of light petroleum and vigorous shaking, the organic phase was separated, dried and an aliquot concentrated on the rotary evaporator. Again, final concentration was carried out with a gentle stream of nitrogen. The samples, made up to volume (1.0 ml) with ethyl acetate, were then analyzed by GC–MS.

Samples that contained TCDD at levels above 100 ppb were re-analyzed by mass fragmentography after being methylated with diazomethane according to the micro-method described by Schlenk and Gellerman[16]. Methylation was carried out in 10% methanol–diethyl ether solution.

Recovery

Recovery experiments were carried out by adding differing amounts of TCDD, ranging from 0.1 to 10 μg, to the hydrolysis reagents, to technical 2,4,5-T, 2,4,5-trichlorophenol or to formulations. These fortification levels correspond to 5–500 ppb for technical materials and 50–5000 ppb for 10% 2,4,5-T formulations.

Determination

For quadrupole mass fragmentography, the mass spectrometer was used to

monitor at unit resolution the molecular ion for TCDD at *m/e* 320. The precision mass meter was adjusted by slowly evaporating a few nanograms of TCDD from the direct insertion probe.

A standard curve was prepared by injecting differing amounts of TCDD (0.5–100 ng) on the GC column and plotting peak heights *versus* amount injected. Quantitation of TCDD in samples was then achieved by comparing their peak heights with the standard curve.

In order to allow injection of up to 50 μl of solution, a venting period of 1 min was used to prevent overloading the MS vacuum system with solvent vapour. Samples prepared by the shortened procedure were analyzed using the dual-column system. An initial venting period of 1 min was used and back-flushing initiated after 4 min, which prevented early- and late-eluting components from entering and contaminating the separator and ion source. This timing period was previously determined by a few standard injections so as to ensure that none of the TCDD is lost through venting and back-flushing.

Confirmation of the identity of TCDD in samples with a suspected content higher than 100 ppb was carried out by MS. A larger aliquot, corresponding to approximately 100 ng of TCDD, was injected and complete mass spectra were recorded. Five mass spectra taken at 15-sec intervals bracketting the retention time of TCDD were sufficient. The spectra were evaluated by comparison of the mass peaks in the region *m/e* 200–500 of scans obtained before, during and after elution of suspected TCDD. The ion intensities of *m/e* 320, 322 and 324 (M^+, four chlorine atoms) and 257 and 259 ($M^+ - COCl$, three chlorine atoms) were compared with that of a TCDD standard. In addition, the spectra were checked for the absence of chlorine containing higher molecular ions that could possibly form fragments at these *m/e* values.

In addition, samples containing large amounts of TCDD were reassessed by mass fragmentography after methylation in order to observe any reduction of the TCDD content caused by this treatment.

RESULTS AND DISCUSSION

The MS data for TCDD indicate intense molecular ions with the characteristic clustering due to chlorine isotopes at *m/e* 320, 322 and 324. The only other ions of some intensity in the higher mass range are at 257 and 259 ($M^+ - COCl$). Quadrupole mass fragmentography was carried out by single ion detection of the ion at *m/e* 320. This ion was used rather than the more intense ion at *m/e* 322 because of possible interference of PCBs. In addition, column bleeding from silicone OV-225 showed a minor signal at *m/e* 322.

The response of the Finnigan 1015D quadrupole mass spectrometer proved to be sufficiently stable over a suitable period of time to allow the use of an external standard for quantitation with acceptable precision. The peak height of the response was found to be linear in the range 0.5–100 ng of TCDD injected. A detection limit of better than 0.5 ng could easily be achieved without any attempt to maximize the response. This corresponds to a level of approximately 1 ppb based on a 20-g sample of technical material. This detection limit was found to be sufficient for

the present work. For the samples investigated, a single GC peak was usually obtained.

Back-flushing and venting the dual-column system was achieved by switching the carrier gas stream and vent by toggle valves outside the heated zones. Such a system has been described by Deans[17] as early as 1965 and more recently by Warner *et al.*[18]. The system allowed the back-flushing of unwanted late-eluting components and prevented them from entering the second, final GC column. With the same system, early-eluting components are vented and do not contaminate the separator and ion source. It is of importance in pesticide analysis that the components of interest are in contact only with glass and not with any metallic parts such as valves. Only components with retention times in a narrow range are determined. Of course, this system is applicable only when a few components are of interest. The use of a first column to cut out unwanted components results in an efficient clean-up and simplifies sample preparations prior to GC analysis. Such systems are easily built, easy to operate and suitable for routine application and automation. In combination with single ion detection, this leads to a high specificity of detection. We are sure that such techniques will gain an increasingly wider application in pesticide analysis.

Recovery experiments for TCDD added to the reagents and carried through all the steps of the procedure ranged from 40% at the 0.1 μg level to 80–100% at the 1–10 μg level. Addition of similar amounts of TCDD to 2,4,5-T and 2,4,5-trichlorophenol led to recoveries of 80–100% at levels corresponding to 50–500 ppb. Recoveries in both 2,4,5-T ester and amine salt formulations were lower (60–80%).

Some results for the content of TCDD in 2,4,5-T are reported in Table I. The samples listed represent the production of a German manufacturer over a period of 6 years. We were told after this investigation that the sample with code 12.8.70 and a content of *ca.* 2000 ppb TCDD was not of German but of American origin.

TABLE I

TCDD CONTENT IN SAMPLES OF 2,4,5-T OBTAINED FROM A GERMAN MANUFACTURER

Sample code	*TCDD content (ppb)*	
	Complete procedure	*Simplified procedure*
7.8.67	300	240
17.7.70	390, 490	520
12.8.70	1840	1950
12.11.70	420	580, 640
12.12.70	50	60
14.9.71	80	60, 70
22.9.72	n.a.*	40
18.9.73	n.a.*	80
Dowicide 2 (2,4,5-trichlorophenol)	2	<3

* Not analysed.

The first column in Table I lists the results obtained according to the original procedure, while the second column lists the results obtained by the shortened procedure.

The results indicate acceptable agreement for duplicate samples and also for the two procedures. The results show that the content of TCDD in technical 2,4,5-T for this manufacturer ranged from 300 to 600 ppb prior to 1971. Since then, the TCDD content decreased to 40–80 ppb, which is about compatible with the 50 ppb specification valid in Switzerland. As a comparison, a single result for a sample of 2,4,5-trichlorophenol (Dowicide 2) is included, which shows a content of TCDD of about 2 ppb.

In addition, a comparison of the results listed in Table I indicates that refluxing samples with methanolic lithium hydroxide does not increase the TCDD content. The simplified method results in a very fast procedure for the analysis of TCDD in technical 2,4,5-T and 2,4,5-trichlorophenol and is suitable for routine analysis in quality control.

Table II lists some typical results obtained by analyzing 2,4,5-T formulations taken from commercial sources in Switzerland in June, 1973. These formulations were analyzed using the complete procedure with refluxing and alumina column clean-up. The results indicate TCDD contents of 20–70 ppb relative to 2,4,5-T. Again, the results are acceptable with our specification.

TCDD in samples with a suspected content above 100 ppb could be confirmed in all instances by MS. Confirmation of TCDD by complete MS analysis on these samples was preferred to multiple ion detection on three or four prominent ions. Scanning the whole mass range from *m/e* 35 to 550 in fixed intervals before, during and after elution of the suspected TCDD allowed the confirmation and identification of TCDD in all those samples, although no GC peak could usually be observed on the total ion monitor, because of a multitude of other components that were eluted from the column at about the same retention time as TCDD. These components

TABLE II

TCDD CONTENT IN 2,4,5-T FORMULATIONS TAKEN FROM COMMERCIAL SOURCES IN SWITZERLAND IN JUNE, 1973

Results are given in ppb relative to 2,4,5-T.

Formulation	*Sample*	*TCDD content*
Ester	Manufacturer I (15% 2,4,5-T)	50
	Manufacturer II (45% 2,4,5-T)	20
Amine salt	Manufacturer III (12% 2,4,5-T)	10, 20
	Manufacturer IV (9% 2,4,5-T)	50, 70
	Manufacturer V (9% 2,4,5-T)	20, 50

could be identified by MS as polychlorinated biphenyls, dibenzofurans and diphenyl oxides.

The presence of polychlorohydroxydiphenyl ethers as possible precursors of TCDD could not be substantiated. This was observed by Rappe and Nilsson[19] for octachlorodibenzo-1,4-dioxin. Our finding is based on the fact that with the samples examined in this study, no significant reduction of the apparent TCDD content occurred after methylation of the neutral part.

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