Bull. Environ. Contam. Toxicol. (1991) 47:822–826

Environmental Contamination and Toxicology

Levels of 3,3′,4,4′-Tetrachloroazobenzene in Propanil Herbicide

Joginder Singh and Ron Bingley

Laboratory Services Division, Food Production & Inspection Branch, Agriculture Canada, Ottawa, Ontario, K1A 0C6, Canada

The occurrence of 3,3',4,4'-tetrachloroazobenzene (TCAB) as a contaminant in commercial samples of 3,4-dichloroaniline (3,4-DCA) and in herbicides like diuron, linuron and propanil, which are derived from 3,4-DCA (Bunce et al. 1974, Hill et al. 1981, Dimuccio et al. 1984, Singh and Bingley 1990) has been well documented. TCAB has been found to induce arylhydrocarbon hydroxylase in mice and chick embryos (Poland et al. 1976), and delayed-wasting syndrome in male rats (Hsia and Kreamer 1985). This compound has also been implicated in the outbreak of chloracne in workers involved in the manufacture of 3,4-DCA (Taylor et al. 1977). Residues of TCAB have been detected in soils treated with the herbicide propanil (Chiska and Kearney 1970). TCAB found in soil can be due to direct spray of TCAB- contaminated herbicides or due to microbial transformation of 3,4-DCA, which is generated in soil from the hydrolysis of herbicides containing the 3,4-DCA moiety, e.g., propanil (Bordeleau et al. 1972, Huges and Corke 1974).

Agriculture Canada, under a program to study microcontaminants in various pesticides, initiated an investigation to determine the TCAB content in propanil herbicide. Twenty-three samples of propanil herbicide obtained from the Canadian marketplace were analysed for the presence of 3,3',4,4'-tetrachloroazobenzene. The level of contamination observed is reported in this paper.

MATERIALS AND METHODS

A stock standard solution of tetrachloroazobenzene (Ultra Scientific, Ontario Canada) was prepared by dissolving 3.5 mg in 100 mL of toluene. A working standard solution containing 35 ug/mL was prepared by diluting this stock solution with hexane.

All solvents were glass distilled (Caledon Laboratories, Ontario, Canada). Woelm Basic Alumina (Activity I) was used as received. Anhydrous sodium sulphate and hydrochloric acid were of reagent grade (Fisher Scientific).

Send reprint requests to Joginder Singh at the above address

The propanil herbicide samples were obtained by the Inspectors of Agriculture Canada from retail outlets in various regions of Canada. A 1.0-g sample of well-mixed herbicide formulation was dissolved in 100 mL of a mixture of methanol, water and concentrated hydrochloric acid (90:9:1 v/v) in a 250-mL separating funnel. This solution was extracted twice with hexane using 25 mL each time. The combined hexane extract was washed with 25 mL of deionized water and then dried by passing it through a plug of anhydrous sodium sulphate in a glass funnel. The dried hexane extract was reduced to 1-2 mL on a rotary evaporator under reduced pressure. This concentrated extract was added to a cleanup column. The glass column (25 cm x 1.4 cm i.d.) with a 250-mL reservoir contained, from the bottom to top, a glass wool plug, a 1.0-cm depth of Ottawa sand, 15-g of Woelm basic alumina Activity I, and a 1.5-cm deep plug of anhydrous sodium sulphate, the entire column was filled with hexane. The TCAB was eluted with 100-mL of 30% methylene chloride in hexane. The eluate was concentrated to about 1-mL on a rotary evaporator under reduced pressure. The concentrate was transferred to a 15-mL graduated centrifuge tube with hexane, and adjusted to an appropriate volume (generally 10 mL). TCAB was analysed on a Varian Vista 6000 gas chromatograph equipped with an electron-Ni63 capture detector (ECD). The 30 m x 0.32 i.d. fused silica capillary column was bonded with a 25 um film thickness of DB-5. The gas chromatograph was interfaced to a Varian 4270 integrator. GC operating conditions were: inlet 250'C, detector 350'C, carrier gas hydrogen, column head-pressure 16 psi; column temperature programming 150' - 280'C at 10'C/min. The levels of TCAB were determined by using the external standard technique.

RESULTS AND DISCUSSION

Twenty-three samples of propanil herbicide were analysed for the presence of 3,3',4,4'-tetrachloroazobenzene. The levels ranged between 1.1 to 30 ppm, as indicated in Table I.

Only three out of the twenty-three samples were contaminated at levels exceeding 10 ppm, the other 87% were at levels equal to or less than 10 ppm. Acidified aqueous methanol was used to dissolve the propanil samples, and TCAB was partitioned into hexane. The acid was added to aqueous methanol to take advantage of the basicity of the NH group in the propanil molecule, and thus minimize its solubility in hexane. Neutral TCAB was partitioned into the hexane phase. Final cleanup was performed on 15-g of basic alumina (Activity I) using 30% methylene chloride in hexane as eluent. The cleanup gave an exceptionally clean extract which was free from any interferences. Typical chromatograms obtained from a sample extract and from the standard TCAB solution are given in Figure 1.

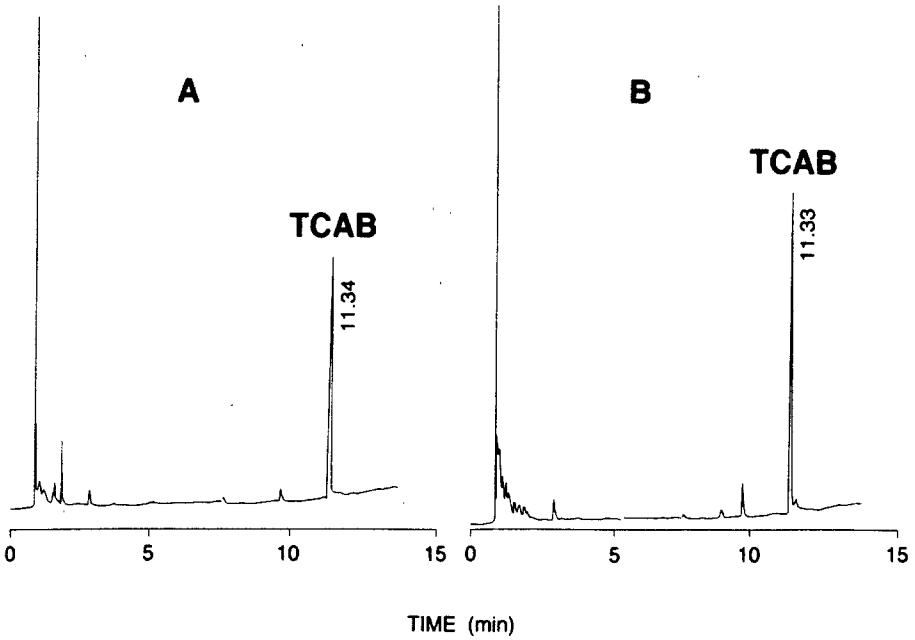

Figure 1. Typical Chromatograms
A, TCAB Standard - B, Sample Extract

Table 1. Levels of 3,3', 4,4'-tetrachloroazobenzene (TCAB) in commercial propanil herbicide

Sample No.	% Technical Ingredient b	TCAB (ppm) a	Sample No.	% Technical Ingredient b	TCAB (ppm) a
1	41.0	13.6	13	37.5	2.1
2	37.5	1.1	14	41.0	1.3
3	41.0	9.8	15	41.0	2.9
4	41.0	11.7	16	41.0	2.4
5	37.5	7.3	17	41.0	6.4
6	37.5	6.3	18	37.5	4.7
7	37.5	10.0	19	41.0	2.8
8	41.0	2.1	20	41.0	7.9
9	41.0	2.3	21	41.0	2.2
10	37.5	1.1	22	37.5	1.4
11	37.5	2.0	23	37.5	30.0
12	37.5	2.1	--	--	--

a The results given are single analysis figures.
b Label guarantee

Table 2. Recovery (%) of TCAB from propanil herbicide

Fortification Level in ppm	Run Number					AVG. (%)	STD. DEV.	C.V. (%)
	1	2	3	4	5			
0.5	76	100	100	108	108	98	13.1	13.4
1	80	96	100	76	112	93	14.8	15.9
5	90	75	80	95	92	86	8.5	9.8
10	87	105	83	94	91	92	8.4	9.1

Table 2 gives the percent recovery obtained on fortified samples of propanil. Samples were fortified at four different levels, i.e., 0.5, 1, 5, and 10 ppm. The average recovery was 98, 93, 86, and 92%, respectively with coefficients of variation of 13.4, 15.9, 9.8 and 9.1%, respectively. As no sample was free of contamination, the recovery study was conducted by the standard addition technique. The herbicide sample was first analysed to establish the actual concentration of TCAB. In the present situation, sample No. 2 in Table I was used for recovery studies; an average of four determinations was used to establish the actual amount present. The present recovery was calculated after subtracting the original amount of TCAB from the results obtained on the fortified sample. The raw data thus obtained forms the contents of Table II. It is appropriate to mention here that it is essential to establish the linear range of the electron-capture detection system (ECD); in the present case it was between 10 to 100 pg. A 10 pg of TCAB injection gave the acceptable 5:1 signal to noise ratio providing integration counts of 31,585 electronic units. Due to this high sensitivity of the electron-capture detector to TCAB, this method is capable of detecting very low levels of TCAB contamination in propanil samples, i.e., in the ppb range. However, due to the lack of a contamination-free propanil sample, the true capability of this procedure to detect extremely low level of TCAB contamination could not be experimentally demonstrated.

REFERENCES

Bordeleau LM, Rosen JD, Bartha R (1972) Herbicide-derived chloroazobenzene residues: Pathway of formulation. J Agr Food Chem 20:573-578.

Bunce NJ, Corke CT, Merrick RL, Bright JH (1979) 3,3', 4,4'-Tetra-chloroazobenzene as a contaminant in commercial propanil. Chemosphere 5:283-284.

Chiska H, Kearney PC (1970) Metabolism of propanil in soils. J Agr Food Chem 18:854

Dimuccio A, Camoni I, Dommarco R (1984) 3,3',4,4'-Tetrachloro-azobenzene and 3,3',4,4'-Tetrachloroazoxybenzene in technical grade herbicides, propanil, diuron, linuron and neburon. Ecotoxicol Environ Safety 8:511-515.

Hill RH, Rollen ZJ, Groce DF, Needham LL (1981) Tetrachloroazobenzene in 3,4-dichloroaniline and its herbicidal derivatives: Propanil, diuron, linuron and neburon. Arch Environ Hlth 36:11-14.

Hsia MTS, Kreamer BL (1985) Delayed wasting syndrome and alternatives of liver gluconeogenic enzymes in rats exposed to the TCDD congener 3,3'4,4'-tetrachloroazoxybenzene. Toxicol Lett 25:247-258.

Hughes AF, Corke CT (1974) Formulation of tetrachloroazobenzene in some Canadian soils treated with propanil and 3,4-dichloro-aniline. Canad J Microbiol 20:35-39.

Poland A, Glover E, Kende AS, DeCamp M (1976) 3,4,3'4'-Tetrachloroazoxybenzene and azobenzene: Potent inducers of Aryl Hydrocarbon Hydroxylas. Science (Washington, DC) 194:627-630.

Singh J, Bingley R (1990) Levels of 3,3'4,4'-Tetrachloroazobenzene in diuron and linuron herbicide formulations. J Assn Off Anal Chem 73:749-51.

Taylor JS, Wuthrich RC, Lloyd KM, Poland A (1977) Chloracne from manufacture of a new herbicide. Arch Dormatol 113:616-619.

Received February 15, 1991; accepted April 4, 1991.