Presence of PCB, DDE and DDT in Human Milk in the Provinces of New Brunswick and Nova Scotia, Canada[1]

by C. J. MUSIAL, O. HUTZINGER
Atlantic Regional Laboratory, National Research Council of Canada, Halifax, Nova Scotia
V. ZITKO
Fisheries Research Board, St. Andrew's, New Brunswick
and
J. CROCKER
Izaak Walton Killam Hospital for Children, Halifax, Nova Scotia

In recent years, numerous references have appeared in the literature on the presence of p,p'-DDT and other chlorinated hydrocarbon insecticide residues in human milk in various countries including Germany (ACKER and SCHULTE 1971, ACKER and SCHULTE 1970, ENGST and KNOLL 1972), Sweden (WESTOO and NOREN 1972), the U.S.A. (DYMENT et al. 1971a, HAGYARD et al. 1973), England (EGAN et al. 1965), Holland (TUINSTRA 1971), Poland (KONTEK et al. 1971), Belgium (HEYNDRICKX and MAES 1969), Russia (GRACHEVA 1970) Canada (RITCEY et al. 1972), and Japan (TAKEDA et al. 1973, NISHIMOTO et al. 1973). Polychlorinated biphenyls have been detected in Germany (ACKER and SCHULTE 1971, ACKER and SCHULTE 1970), Sweden (WESTOO and NOREN 1972), Japan (NISHIMOTO et al. 1973), and Colorado, U.S.A. (SAVAGE et. al. 1973). In an earlier study, PCB was not detected in human milk in Texas and New Guinea (DYMENT et al. 1971b). The purpose of the present study was to investigate the levels of PCB, DDE and DDT contamination in human milk from two of Canada's Atlantic Provinces, New Brunswick (N.B.) and Nova Scotia (N.S.). Several samples of commercially prepared infant formulas were also analyzed.

Materials and Methods

Six lactating mothers from N.B. and nine from N.S. were the subjects of the study. Milk samples from N.B. were obtained by manual expression and from N.S. by pump into chemically clean glass containers. All samples were stored at -20° until time of assay.

Samples of breast milk (4-6 g) and of prepared formulas (6-8 g) were dispersed in anhydrous sodium sulfate which had been previously washed free of electron-capturing impurities with Fisher Pesticide Grade hexane. Each mixture was extracted with hexane in a Soxhlet apparatus for 1-1/2 hours and the extract concentrated to 1 ml at room temperature on a rotary vacuum evaporator.

NRCC No. 13692.

Bulletin of Environmental Contamination & Toxicology.
Vol. 12, No. 3 © 1974 by Springer-Verlag New York Inc.

The procedure of HOLDEN and MARSDEN (1969) was used to clean up the extract and separate PCB from most of the chlorinated hydrocarbon insecticides. Alumina (Fisher A-540) was activated at 800°C overnight and deactivated by the addition of 5% distilled water. Silica gel (Merck #7754) was activated overnight at 130°C and deactivated by the addition of 3% distilled water (ARMOUR and BURKE 1970). The silica gel was found to be contaminated with electron-capturing impurities and it was necessary to wash it with redistilled ether followed by hexane. The performance of silica gel was variable and the elution pattern of PCB and insecticides had to be checked for each new batch of silica which was washed and activated. Columns 7 mm I.D. × 60 mm long, plugged with a piece of pre-washed glass wool and filled with 2 g of either alumina or silica, were used. The concentrated extract (1 ml) was applied to the alumina column and washed in with an additional 1.5 ml hexane. The column was percolated with hexane until 20 ml of effluent were collected. The effluent was concentrated to 1 ml in a rotary vacuum evaporator at room temperature and applied to the silica column, washed in with an additional 1.5 ml of hexane, and the column was washed with hexane. Depending on the batch of silica gel, PCB and DDE were eluted with 6-8 ml of hexane (Fraction 1), followed by p,p'-DDT, 14-16 ml of hexane (Fraction 2). Recoveries using these techniques have been shown to be essentially complete (HOLDEN and MARSDEN 1969, ARMOUR and BURKE 1970). In some cases the extract did not completely clean up on alumina but the residual lipid (2-3%) did not pass through the silica column.

The assurance of absolute chemical cleanliness of all glassware, solvents, etc. used in these analyses cannot be overemphasized. Each new batch of any reagent must be checked for electron-capturing impurities prior to use. When extreme care was given to these details, it was possible to run a blank which showed no interfering peaks when the final injection was made into the gas chromatograph. Where breast pumps were used to collect the samples, these were checked for the presence of contaminants by using a sample of previously analyzed warm breast milk, after which treatment the residue levels of the milk were not appreciably altered.

The effluent fractions were concentrated just to dryness on a rotary vacuum evaporator at room temperature and made up to known volume for injection into the gas chromatograph. A Hewlett-Packard Model 5750 chromatograph fitted with a Nickel 63 detector was used. The column was 6' × 6 mm glass packed with 4%

SE-30 on Chromosorb WAW-DMCS, operated at 200°C with nitrogen flow of 60 ml/min. Injection port - 240°C, detector - 265°C at pulse interval of 150 μsec.

The detection limit (minimum detectable levels) on wet weight basis are: PCB-Aroclor 1254 (0.01 μg/g); p,p'-DDE (0.003 μg/g) and p,p'-DDT (0.002 μg/g).

The computerized GC/MS data were obtained on a Finnigan Model 3100D instrument and a Finnigan Model 6000 data system using a glass jet-type separator. The following conditions apply. Mass spectrometry: electron energy, 70 eV; filament current, 400 microamps; analyzer pressure, 1×10^{-5} torr; analyzer temp., 90°C; scan time, 3 seconds. Gas chromatography: column, 5' × 2 mm ID glass packed with 3% OV-1 on 100/120 mesh gas chrom.; column temp, 160-230°C programmed at 6°C/min; carrier gas, helium at 20 cc/min; injector temp., 250°C; interface temp., 250°C.

Results and Discussion

In Table 1 the results are listed of the analyses for PCB, p,p'-DDE and p,p'-DDT for the two areas concerned. The levels of p,p'-DDE and p,p'-DDT are seen to be somewhat higher in N.B. while PCB levels are approximately the same.

It should be noted that, as compared to the quantitation of DDE and DDT, the quantitation of PCB is somewhat involved because of its multicomponent nature. In the case of the human milk samples, six peaks whose retention times and relative heights corresponded to those of Arochlor 1254 were used for quantitation, out of a possible 10 peaks of an Arochlor 1254 sample as supplied by Monsanto. The peaks used for 1254 quantitation all had retention times greater than DDE, which elutes from the silica column with PCB. The quantitative interference of PCB with DDE is usually very small.

Only trace quantities of p,p'-DDT and p,p'-DDE (< 0.003 μg/g wet weight) were detectable in the prepared infant formulas. In none of the prepared infant formulas was PCB as Aroclor 1254 detected. However, PCB whose GLC pattern showed resemblance to the Aroclor 1242 type was detected, at a level of 0.088 ppm in the whole (undiluted) formula (mean of 5 samples). Peaks corresponding to Aroclor 1242 were also detected in all breast milk samples but these were not quantified because the peak pattern was not clear. The major 1242 components elute from the SE-30 column

TABLE 1

PCB, p,p'-DDE and p,p'-DDT in Human Milk in New Brunswick (N.B.) and Nova Scotia (N.S.)

	PCB, as Aroclor 1254 p.p.m.		p,p'-DDE p.p.m.		p,p'-DDT p.p.m.		%
	Whole milk	Lipid	Whole milk	Lipid	Whole milk	Lipid	Lipid
N.B.							
1	0.016	1.69	0.037	3.99	0.016	1.77	0.92
2	0.015	1.07	0.068	4.78	0.030	2.11	1.43
3	0.030	1.68	0.031	1.78	0.010	0.57	1.77
4	0.022	1.38	0.017	1.12	0.006	0.39	1.56
5	0.020	1.26	0.020	1.26	0.008	0.49	1.61
6	0.028	2.10	0.036	2.74	0.006	0.41	1.32
MEAN	0.022	1.53	0.035	2.61	0.013	0.96	1.44
S.D.	±0.007	±0.37	±0.018	±1.51	±0.009	±0.77	
N.S.							
1	0.032	2.28	0.040	2.83	*	*	1.40
2	0.019	1.71	0.013	1.21	*	*	1.08
3	0.018	1.63	0.031	2.75	0.011	0.98	1.12
4	*	*	0.009	1.32	*	*	0.62
5	0.011	1.31	0.018	2.23	0.006	0.66	0.81
6	*	*	0.018	0.98	*	*	1.79
7	0.017	2.38	0.015	2.14	0.004	0.56	0.69
8	0.012	2.46	0.012	2.08	0.002	0.45	0.51
9	0.020	1.22	0.011	0.70	0.008	0.41	1.70
MEAN	0.018	1.86	0.019	1.80	0.006	0.61	1.08
S.D.	±0.007	±0.52	±0.010	±0.77	±0.002	±0.23	

* Present, not quantified.

TABLE 2

Comparison of PCB, p,p'-DDE and p,p'-DDT Levels in Human Milk in Various Countries

Country	Year	PCB, p.p.m.		p,p'-DDE, p.p.m.		p,p'-DDT, p.p.m.		Ref.
		Whole milk	Lipid	Whole milk	Lipid	Whole milk	Lipid	
GERMANY	1970*	0.103	3.5	0.081	2.7	0.031	1.1	2
SWEDEN	1971-72	0.025		0.059	1.9	0.020	0.63	4
USA (TEXAS)	1971*			0.084		0.023		5
ENGLAND	1963-64			0.073		0.045		7
HOLLAND	1969			0.03	1.9	0.016	1.0	8
POLAND	1971*			0.20	8.9	0.082	3.9	9
BELGIUM	1969*			0.072		0.047		10
RUSSIA	1970*			0.101		0.097		11
CANADA (Atlantic Provinces)	1967			0.0093	3.559	0.033	1.206	12
CANADA								
N.B.	1972	0.022	1.53	0.035	2.61	0.013	0.96	THIS
N.S.	1972	0.018	1.86	0.019	1.80	0.006	0.61	STUDY
COLORADO	1973*	0.04						15
JAPAN	1972*	0.00-0.03		0.009-0.077		0.006-0.033		13,14

*Year published.

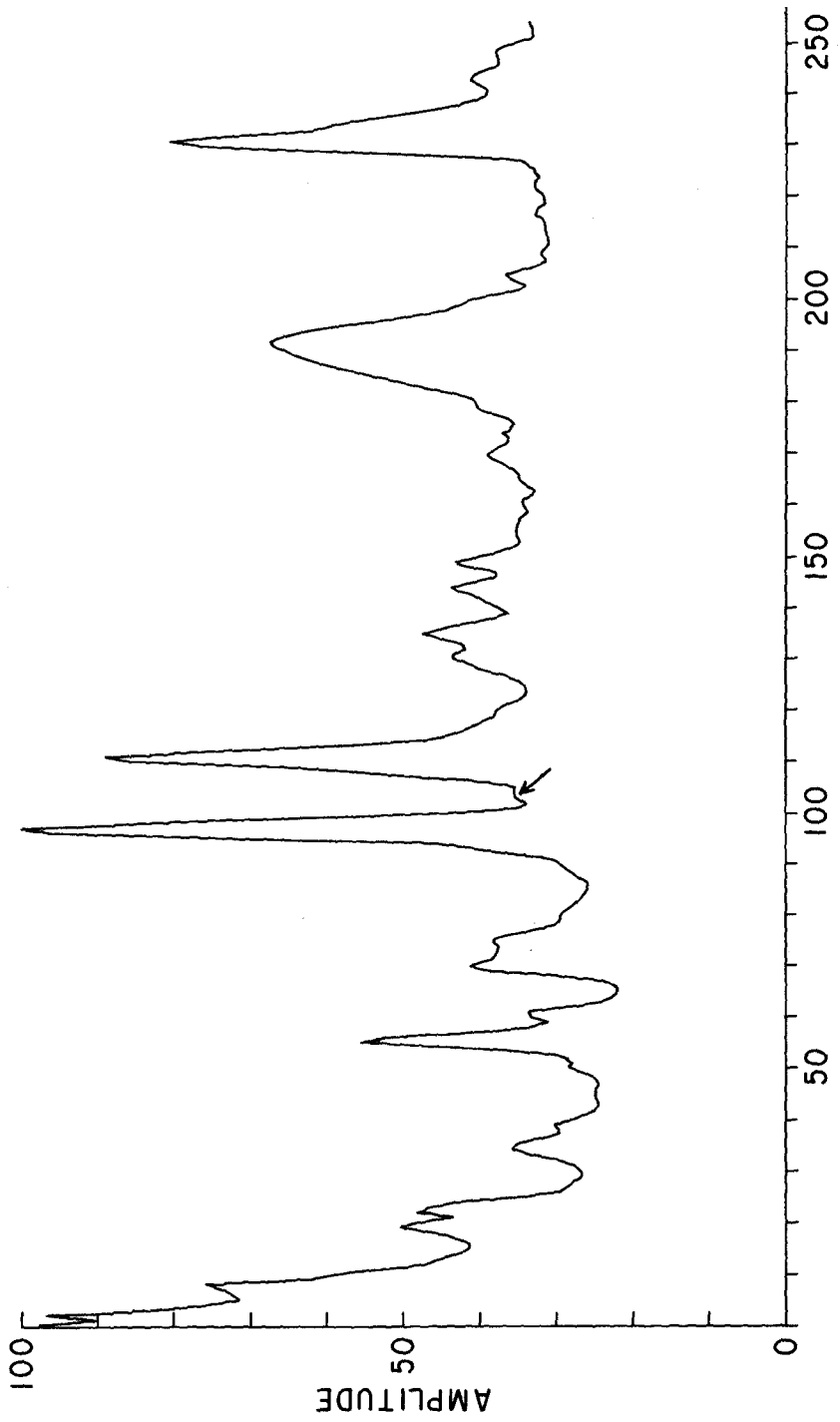

Fig.1 Total ion chromatogram of cleaned-up breast milk lipids, arrow indicates DDE peak.

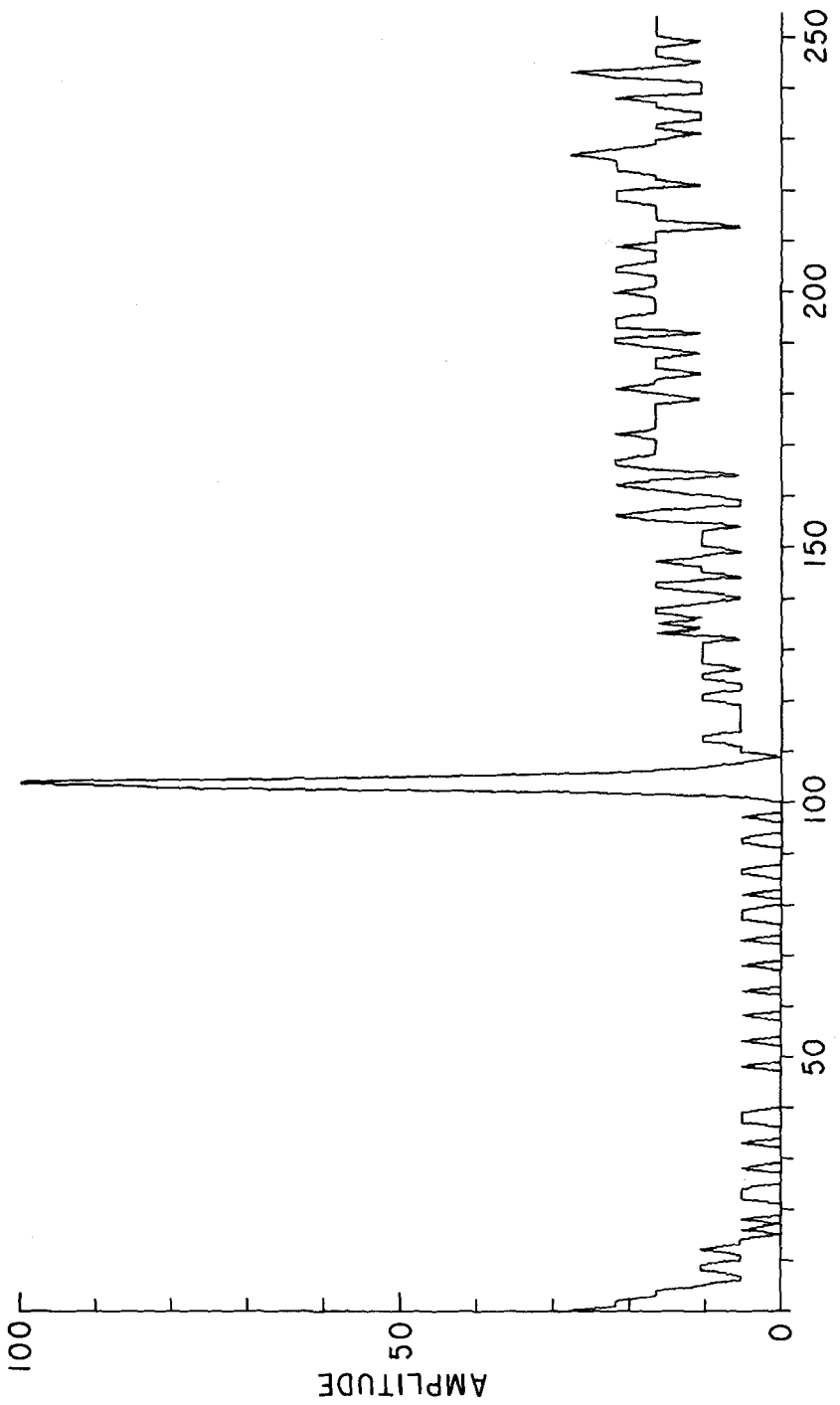

Fig. 2 Limited mass search for m/e 316

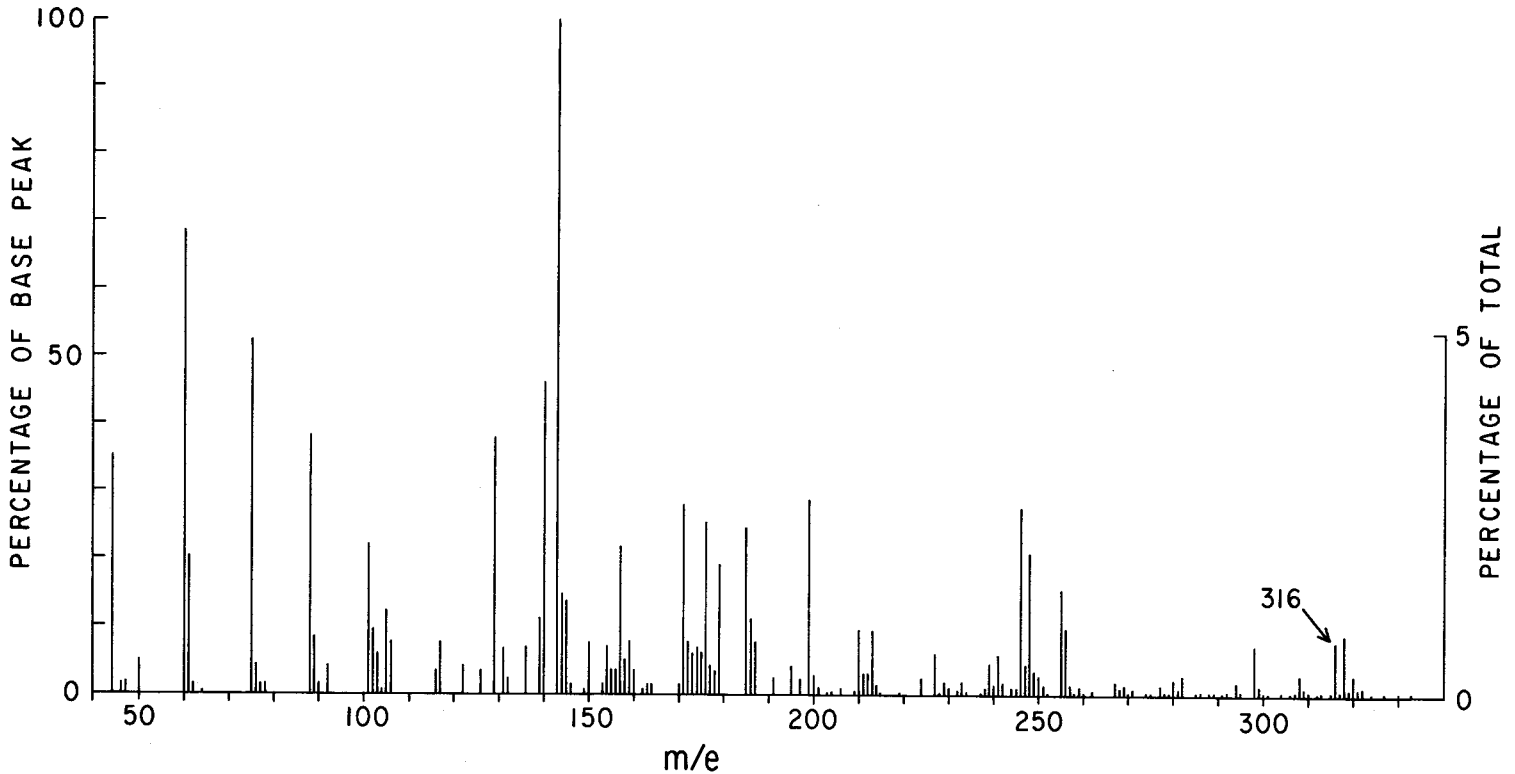

Fig.3 Mass Spectrum number 104 (DDE)

before p,p'-DDE and the six 1254 peaks which were used for quantitation. Thus, interference of 1242 with 1254 quantitation is negligible.

It is interesting to note that the prepared formulas contained only trace amounts of p,p'-DDT and p,p'-DDE.

In Table 2, PCB, p,p'-DDE and p,p'-DDT levels in human milk of various countries are given. PCB levels in Canada compare closely with the Swedish results and are lower than those reported in Germany and the U.S.A. (Colorado). DDE and DDT levels as reported in this study are comparable to those reported for Holland, lower than those reported for the other countries listed, and lower than those previously reported for the Canadian areas studied.

The presence of PCB and DDE in several of the samples analyzed was confirmed by high resolution mass spectrometry (cf. JAMIESON et al. 1973) and computerized GC/MS (cf. BONELLI 1972). An example of the latter is shown in Fig. 1 (total ion chromatogram of cleaned-up breast milk lipids); Fig. 2 (limited mass search for m/e 316); Fig. 3 (mass spectrum of DDE).

Summary

PCB, p,p'-DDE and p,p'-DDT residue levels were measured in human milk from New Brunswick and Nova Scotia. Preliminary results indicate that the levels of p,p'-DDT and p,p'-DDE are higher in the former area, possibly because of the wider use of insecticide sprays in the past. It should be emphasized that because of the limited number of samples analyzed, further work must be done before a significant difference in these areas can be established.

Acknowledgements

The authors wish to thank the Canadian Department of Health and Welfare for financial support (Project No. 603-7-27). Sincere thanks are also due to Dr. Bonelli and Mr. Knight for the computerized GC/MS data.

References

1. ACKER, L., and E. SCHULTE: Ernährungsforschung 16, 559 (1971).
2. ACKER, L., and E. SCHULTE: Deut. Lebensm. Rundsch. 66, 385 (1970).
3. ENGST, R., and R. KNOLL: Pharmazie 27, 526 (1972).

4. WESTOO, G., and K. NOREN: Var Foeda 24, 41 (1972).
5. DYMENT, P. G., L. M. HEBERTSON, W. J. DECKER, E. D. GOMES, and J. S. WISEMAN: Bull. Environ. Contam. Toxicol. 6, 449 (1971a).
6. HAGYARD, S. B., W. H. BROWN, J. W. STULL, F. M. WHITING, and S. R. KEMBERLING: Bull. Environ. Contam. Toxicol. 9, 169 (1973).
7. EGAN, H., R. GOULDING, J. ROBURN, and J. O. G. TATTON: Brit. Med. J. 2, 66 (1965).
8. TUINSTRA, L. G. M. T.: Neth. Milk Dairy J. 25, 24 (1971).
9. KONTEK, M., S. KUBACKI, S. PARADOWSKI, and B. WIERZCHOVIECKA: Pediat. Pol. 46, 183 (1971).
10. HEYNDRICKX, A., and R. MAES: J. Pharm. Belg. 24, 459 (1969).
11. GRACHEVA, G. V.: Vopr. Pitan 29, 75 (1970).
12. RITCEY, W. R., G. SAVARY, and K. A. MCCULLY: Can. J. Pub. Health 63, 125 (1972).
13. TAKEDA, M. et al.: Shokuhin Eiseigaku Zasshi 13, 422 (1972); Chem. Abstr. 78, 38975 (1973).
14. NISHIMOTO, T., M. UYETA, S. TAUE, and K. CHIKAZAWA: Igaku No Ayumi 82, 574 (1972); Chem. Abstr. 78, 14573 (1973).
15. SAVAGE, E. P., J. D. TESSARI, J. W. MALBERG, H. W. WHEELER, and J. R. BAGBY: Bull. Environ. Contam. Toxicol. 9, 222 (1973).
16. DYMENT, P. G., L. M. HEBERTSON, E. D. GOMES, J. S. WISEMAN, and R. W. HORNABROOK: Bull. Environ. Contam. Toxicol. 6, 532 (1971b).
17. HOLDEN, A. V., and K. MARSDEN: J. Chromatogr. 44, 481 (1969).
18. ARMOUR, J. A., and J. A. BURKE: J. Ass. Offic. Anal. Chem. 53, 761 (1970).
19. JAMIESON, W. D., O. HUTZINGER, S. SAFE, J. F. S. CROCKER, and V. ZITKO: Proceedings of the 21st Annual Conference of the American Society of Mass Spectroscopists, San Franciso, 1973.
20. BONELLI, E. J.: Anal. Chem. 44, 603 (1972).