Chlordane Residues in Milk and Fat of Cows Fed HCS 3260 (High Purity Chlordane) in the Diet[1]

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Whereas technical chlordane is a complex mixture (MARTIN 1968), HCS 3260 is a high purity form of chlordane containing over 95% α-and γ-chlordane (Velsicol Chem. Corp. Bull. # EL-1-3 1970). An Emulsifiable concentrate formulation of technical chlordane was shown to consist of at least 9 components with α- and γ-chlordane each accounting for about 14% of the total active ingredients. An EC formulation of HCS 3260 contained 74% α-chlordane and 24% γ-chlordane (DOROUGH and PASS 1972; DOROUGH and SKRENTNY 1972).

The higher concentrations of α-and γ-chlordane in HCS 3260 necessitate that new studies be conducted on the residual nature of this insecticide. It is particularly important considering that oxychlordane, a metabolite formed from both chlordane isomers, recently has been reported (LAWRENCE and BARROW 1970; SCHWEMMER et al 1970; POLEN et al 1971; STREET and BLAU 1972). While the precursors are present in technical chlordane, their lower concentration may partially explain why oxychlordane was not recognized earlier. Moreover, oxychlordane may have been reported as heptachlor epoxide, since the 2 compounds cannot be separated by many of the more common gas chromatographic (glc) systems used for chlorinated hydrocarbon residues.

Because oxychlordane was first isolated from the milk of cows fed alfalfa treated with technical chlordane (LAWRENCE and BARROW 1970) and comparable data were unavailable with HCS 3260, our laboratory, in cooperation with scientists at Velsicol Chemical Corporation, performed an experiment to determine if residues appeared in the milk and tissues of dairy cows fed a diet containing low levels of chlordane and/or its metabolites.

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The source of the insecticides was sugar beet pulp processed from plants grown in soil treated 1 week prior to planting with either HCS 3260 at rates of 2.5, 5, 10 or 20 lb AI/a or chlordane at 10 lb AI/a. Processing of the beets was according to standard commercial procedures and the dried sugar beet pulp was fed to the cows. For each treatment rate, 2 pounds of pulp were fed to 1 Holstein and 1 Jersey cow at the morning milking for a period of 30 days. In addition, each animal received a dairy concentrate at each milking and was provided alfalfa hay ad libitum. Table 1 shows the insecticides and their level in the beet pulp fed to the cows and their concentration when considered on the total estimated daily food consumption of the animal. The milk was analyzed throughout the 30-day feeding period and for another 30 days after the cows were returned to a normal ration.

Milk was virtually free of any of the insecticides for which analyses were made (α-, γ-chlordane, oxychlordane, heptachlor, heptachlor epoxide). Trace quantities of what appeared to be oxychlordane were observed in milk of cows fed pulp from the HCS 3260, 10 and 20 lb/a treatments. However, the levels were less than the lower limit of sensitivity of the method, 0.01 ppm in the milk fat, and the identity of the residue was not confirmed. Samples of the liver, kidney and muscle, taken 30 days after terminating the feeding of the sugar beet pulp, were free of detectable residues (< 0.01 ppm). Fat from cows fed pulp from the 10 and 20 lb/a treatments contained low levels (0.016 and 0.024 ppm) of a compound having the same glc characteristics as oxychlordane, but otherwise no residues were detected. The appearance of a compound suspected of being oxychlordane in the milk and fat of cows fed sugar beet pulp from the HCS 3260 treatments prompted additional investigation of this insecticide in lactating cows. Analytical procedures used in the sugar beet study were essentially the same as those described below.

EXPERIMENTAL

Treatment and Sampling. Three Holstein cows selected from the University of Kentucky Dairy Farm were used in this study. Each animal weighed approximately 1400 lbs. and was producing about 50 lbs. of milk per day. Technical grade HCS 3260 was administered to the cows via a gelatin capsule after each morning milking for 60 days. The amounts of insecticide in the capsules were equivalent to that consumed by animals receiving 50 lbs/day of feed containing either 1 ppm, 10 ppm or 100 ppm HCS 3260. The low level was 10-fold greater than the highest level fed to the cows in the sugar

beet study (Table 1). Equal portions of whole milk from the morning and evening milking were combined daily and frozen until analyzed. In the case of the cow fed 100 ppm HCS 3260, morning and evening milk samples also were saved separately to determine if differences existed in the levels of insecticide residues present. Fat biopsis were taken from each animal 30 and 60 days after treatment was initiated, and then 30 days after the HCS 3260 source was removed.

Sample Preparation. Extraction and cleanup of milk and fat for chlordane analysis were conducted using a modification of Velsicol Chemical Corporation's Analytical Method No. AM 0509. A 25-g aliquot of whole milk was mixed with 50 ml. of 95% ethanol and then extracted 3 times with 50-ml. portions of a 1 to 1 mixture of petroleum ether and ethyl ether. The extracts were combined, washed twice with 150 ml. of 5% sodium sulfate solution, and the ether phase dried with anhydrous sodium sulfate. The extract was concentrated to the milk fat residue on a rotary evaporator and the weight of the extracted fat determined.

After quantitatively transferring the milk fat to a aluminum oxide column (20 x 400 mm. glass column containing 20 g. of aluminum oxide deactivated with 5% water and pre-washed with 50 ml. of hexane), with 5 ml. of hexane, the insecticides were eluted with 50 ml. of hexane. The hexane was concentrated to a volume suitable for gas chromatographic analysis. The body fat was prepared for analysis by dissolving 1 g in warm hexane and then proceeding with the aluminum oxide column procedure as described for the milk fat.

Gas Chromatography. A Varian Aerograph series 1700 gas chromatograph equipped with an electron capture detector was used in these studies. The glass column, 2 mm. x 6 ft., was packed with 4% SE30 and 6% QF1 (1:1 mixture) on Anakrom ABS, 80/90 mesh, and operated at 200°C. Injector and detector temperatures were 215°C and 210°C, respectively; the carrier gas was nitrogen at 18 psi. Retention times, in minutes, for the compounds considered for quantitation were as follows: heptachlor, 6.0; oxychlordane, 10.4; heptachlor epoxide, 11.1; α-chlordane, 13.4; γ-chlordane, 12.1.

Mass Spectrometry. The material in the milk and fat having the same glc characteristics as oxychlordane was further analyzed by mass spectrometry. For these analyses, samples from the 100 ppm feeding level were utilized. Mass spectra were obtained on a Finnigan Model 1015C GC/MS system. Analyses were performed on extracts prepared for standard glc evaluations and on

TABLE 1.

Levels of heptachlor (H), heptachlor epoxide (HE), α-chlordane (AC), and γ-chlordane (GC) in the diets of cows fed sugar beet pulp derived from plants grown in soils treated with chlordane or HCS 3260.[a]

Sample, treatment and rate[b]	PPM in sugar beet pulp					PPM based on total daily food intake[c]				
	H	HE	AC	GC	TOTAL	H	HE	AC	GC	TOTAL
Control	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Chlordane, 10 lb/a	.024	.066	.229	.127	.446	.001	.003	.009	.005	.018
HCS 3260 20 lb/a	0	0	1.901	.446	2.346	0	0	.076	.019	.095
HCS 3260 10 lb/a	0	0	1.279	.276	1.555	0	0	.051	.011	.062
HCS 3260 5 lb/a	0	0	.625	.190	.815	0	0	.025	.008	.033
HCS 3260 2.5 lb/a	0	0	.283	.048	.332	0	0	.011	.002	.013

[a] Cows fed 2 lbs. of pulp at morning milking.

[b] Designates the insecticide and rates applied to soil at the time sugar beets were planted.

[c] Using 50 lbs. as an estimated total food consumption/cow/day.

Limit of sensitivity of method = 0.005 ppm for individual components.

samples collected from the glc system described in the above section.

RESULTS AND DISCUSSION

Recovery. Recovery of the chlordanes from control milk and fat exceeded 80% and usually ran above 92%. There were no differences in the recoveries of the 3 compounds. The control samples were free of materials which interfered with the quantitation of the chlordanes.

Milk Fat. Detectable quantities of α-chlordane, γ-chlordane and oxychlordane were present in the milk fat of cows fed HCS 3260. Fig. 1 shows the total of these residues during the 60-day period the cows were on the insecticide treatment, and for an additional 60 days after being returned to untreated feed. Total HCS 3260 residues in the milk fat rose rapidly when the animals were placed on the insecticide-containing diet. At the 1.0 ppm feeding level, total residues leveled off at about 0.5 ppm after 10 days. However, approximately 35 and 45 days were required before the HCS 3260 residues plateaued at approximately 2.5 ppm and 5.0 ppm when the cows were fed 10 ppm and 100 ppm of the insecticide in the diet, respectively.

Removal of the HCS 3260 from the diet resulted in a marked decline of chlordane residue in the milk fat (Fig. 1). By 7 days, the residues were less than one-half the maximum levels reached while the animals were on the treated diet. Thereafter, the rate of disappearance was much slower and only very little decrease was evident after 14 days.

The major product in the milk fat was identified as oxychlordane (Table 2). Its mass spectrum was identical to that of authentic oxychlordane. While the animals were on treatment, oxychlordane accounted for 70 to 75% of the total chlordane residues in the milk, its relative concentration increasing with time. Even after a plateau in the levels of residues was achieved, the concentration of oxychlordane, relative to the α- and γ-isomers, continued to increase slowly. Generally, α-chlordane accounted for about 20% of the total residues while the remaining 5 to 10% was as γ-chlordane.

While the concentration of all residues dropped rapidly during the week following termination of treatment, oxychlordane levels stabilized after about 2 weeks and remained relatively constant for the duration of the experiment. It accounted for essentially 100% of the total chlordane residues in milk after the HCS 3260 had been removed from the diet for 10 days.

TABLE 2.

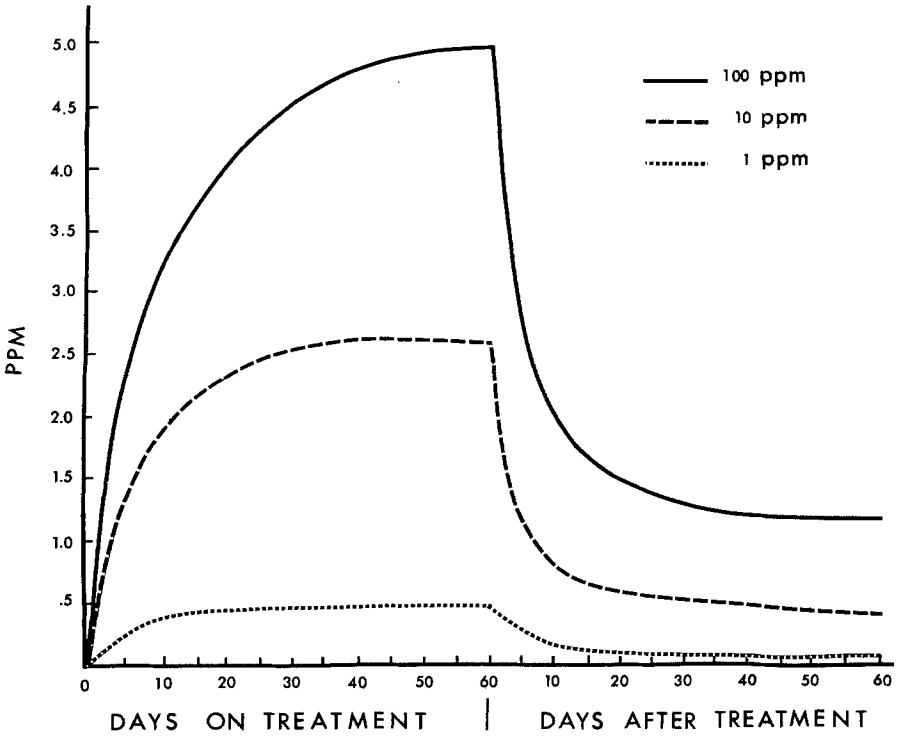
Nature and level of residues in milk of cows fed HCS 3260 in the diet for 60 days.

HCS 3260 in diet and residues in milk	PPM in milk fat at indicated level of HCS 3260 in diet[a]									
	Days on treatment					Day after treatment terminated				
	3	7	15	30	60	1	7	15	30	60
1.0 PPM										
α-chlordane	0.07	0.12	0.08	0.14	0.11	0.07	0.08	< 0.01	< 0.01	< 0.01
γ-chlordane	.03	.05	.03	.03	.03	.03	.03	< .01	< .01	< .01
Oxychlordane	.09	.15	.22	.26	.34	.26	.18	.11	.08	.10
Total	.19	.32	.33	.43	.48	.36	.29	.11	.08	.10
10.0 PPM										
α-chlordane	.24	.32	.42	.55	.44	.55	.05	< .01	< .01	< .01
γ-chlordane	.08	.10	.17	.16	.11	.13	<.01	< .01	< .01	< .01
Oxychlordane	.55	1.11	1.51	1.82	2.09	1.56	.76	.62	.68	.47
Total	.87	1.53	2.10	2.53	2.64	2.24	.81	.62	.68	.47
100.0 PPM										
α-chlordane	.51	.48	.80	.99	.78	.65	.54	< .01	.03	.02
γ-chlordane	.18	.28	.39	.45	.28	.33	.19	.01	.01	.01
Oxychlordane	1.13	2.22	2.57	3.14	3.79	3.73	1.97	1.53	1.34	1.23
Total	1.82	2.98	3.76	4.58	4.85	4.71	2.51	1.53	1.38	1.26

[a] Butterfat content of milk averaged 3.6%.

Analysis of the morning and evening milk samples from the cow fed 100 ppm HCS 3260 showed that the residues were generally lower in the evening samples. However, the differences were small and the ratios of individual chlordane residues were approximately the same.

FIGURE 1



Total chlordane residues (α- and γ-chlordane and oxychlordane) in the milk fat of cows fed 1, 10 and 100 ppm HCS 3260 in the diet for 60 days.

Fat. At the 1 ppm level, total chlordane residues in the fat increased from 0.24 ppm after 30 days of feeding to 0.47 ppm after 60 days (Table 3). Thirty days after treatment was terminated, the residues remained at essentially the same level, 0.45 ppm. This general pattern was evident in the fat of cows fed 10 ppm and 100 ppm of HCS 3260, with total chlordane residues being on the order of 1.2 to 1.5 ppm and 2.6

TABLE 3.

Nature and levels of residues in fat biopsis of cows fed HCS 3260 in the diet for 60 days.

HCS 3260 in diet and residues in fat	PPM at indicated stage of experiment[a]		
	Days of treatment		30 days after treatment terminated
	30	60	
1.0 PPM			
α-chlordane	0.05	0.11	0.04
γ-chlordane	.03	.04	.02
Oxychlordane	.16	.32	.39
Total	.24	.47	.45
10.0 PPM			
α-chlordane	.28	.20	.06
γ-chlordane	.09	.06	.02
Oxychlordane	1.03	.92	1.45
Total	1.40	1.18	1.53
100.0 PPM			
α-chlordane	.51	.60	.20
γ-chlordane	.18	.23	.06
Oxychlordane	1.96	3.14	2.72
Total	2.65	3.97	2.98

[a] Limit sensitivity of method - 0.01 ppm for individual components.

and 4.0 ppm, respectively. Oxychlordane, its identity confirmed by mass spectrometry, accounted for 70 to 80% of the total chlordane residues in the fat during the time the animals were on treatment, and from 85 to 95% in the 30-day post-treatment samples. In these latter samples, concentrations of the total residues were of the same magnitude as in that fat of the animals while on the HCS 3260 treatment. Thus, no estimate of the residual life of oxychlordane in the body fat of these animals could be made from these data.

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