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Fate of Toxaphene, Methyl Parathion, and Chlordimeform Combinations in the Mouse

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The metabolism and fate of toxaphene in mammalian systems have been recently studied. CASIDA et al. (1974), CROWDER and DINDAL (1974), OHSAWA et al. (1975), POLLOCK and KILGORE (1976), and SALEH et al. (1977) reported reductive dechlorination and dehydrochlorination reactions involved in the metabolism of toxaphene in rats. These reactions were also demonstrated in vitro for rat liver microsomes under anaerobic conditions (KHALIFA et al. 1976). Besides the dechlorination reactions, the importance of oxidative mechanisms has also been shown. The metabolism of toxaphene and two of toxaphene's toxic components, Toxicant B (2, 2, 5-endo, 6-exo, 8, 9, 10-heptachlorobornane) and Toxicant C (2-endo, 3, 3, 5, 6-exo-8, 9, 10, 10-nonachlorobornane) were investigated in rats by CHANDURKAR and MATSUMURA (1979a, b) both in vivo and with rat liver preparations. Polar hydroxyl and acidic compounds and water soluble conjugates resulted from the NADPH-dependent mixed function oxidase system.

Toxaphene (T) is commonly formulated with methyl parathion (MP), usually in a 2:1 (T:MP) mixture, for certain insect pests of cotton. Chlordimeform (C) is often added to this formulation (2:1 : 1/4 or 1/8 C) for additional control. WARE et al. (1979) determined that the function of T in the former formulation was to increase the initial deposit and extend the residual life of MP. The effect of these other compounds on T's metabolism and excretion can be questioned. Therefore, to determine if combinations of MP-T-C affect the excretion, retention, or mortality of any of the individual compounds when orally fed to mice, the following experiment was conducted in 3 parts: (1) Toxaphene - T alone and in combination with the other compounds. The combinations were T-MP, T-C, and T-MP-C, (2) Chlordimeform - C-T, C-MP, and C-T-MP, (3) Methyl parathion - MP and MP-T.

MATERIALS AND METHODS

Toxaphene: Male Swiss mice, 16-17 weeks old and weighing an average of 40 g, were orally dosed via a syringe and feeding tube attached to a motor driven microapplicator with 1.0 mg (25 mg/kg) ^{36}Cl-toxaphene (42 μCi/g; Boots-Hercules, Inc.) in 0.16 mL corn oil. For the T-MP combination, the dose included 0.5 mg MP (12.5 mg/kg), TC included 0.125 mg C (3.25 mg/kg), and T-MP-C had 0.5 mg MP and 0.125 mg C added to the 1.0 mg T. For excretion studies, 3 replicates of 4 mice each were placed into glass metabolism chambers following dosing (HALLADAY 1973). The metabolism chambers provided for separate collection of urine and

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feces, and were maintained in an air conditioned environment (22-25°C, 50% RH; light:dark cycle - 11:13). The animals were provided laboratory chow and water ad libitum. Urine and feces were collected and mortality counts made at 3, 6, 12, 24, 48, 72, 96, 168, and 192 h. Feces and urine were stored at - 20°C to await further analysis. One mouse from each replicate was sacrificed at 192 h and frozen to be analyzed for tissue retention. The remaining mice in each replicate were given an additional dose; these animals were referred to as "redosed". Urine and feces were collected in the previously described manner. One mouse from each replicate of redosed animals was sacrificed at the last sample time and another on Day 33 following redose, and both retained for tissue storage determination. At each sampling period cages were rinsed to remove any urine residues; the rinses were frozen until analyzed.

Feces samples were thawed, air dried, weighed, ground to a powder, and two-50 mg subsamples digested in 3 mL of TS-1^R tissue solubilizer using heat to aid digestion. Bleaching was accomplished with 0.3 mL benzoyl peroxide (200 mg/mL toluene). Tissues (brain, liver, lipid, kidney, skeletal muscle, and testes) were weighed and 50 mg subsamples digested in 3 mL of TS-1^R. Radioassay was performed with scintillation counting techniques; quench was corrected using the external standard method.

Chlordimeform: All methods employed and dosages applied in this part of the experiment were identical to those described under Toxaphene. The only differences were the dosing combinations (C, C-T, C-MP, and C-T-MP) and the use of ^{14}C-chlordimeform (15.3 μCi/mg; Ciba-Geigy Corp.).

Methyl Parathion: Methods employed and dosages applied were the same as previously described with the following exceptions. Only MP and the T-MP were evaluated, with neither compound being radiolabelled. Urine and feces were collected at 3, 6, 12, 24, 48, and 96 h only, and no redose was made. Four mice from each treatment were retained for tissue analysis, and only liver, kidney, and lipid were sampled. Urine was analyzed for para-nitrophenol (PNP) by electron capture gas chromatography after CRANMER (1970). One gram subsamples (total sample if <1.0 g) of feces and tissues were extracted in hexane, dried through sodium sulfate and concentrated to 10 mL. Fecal extracts from each time period were pooled and concentrated to one-10 mL sample. Lipid samples were partitioned between acetonitrile and hexane (4:1) after the initial hexane extraction, dried, reconstituted in hexane, dried through sodium sulfate, and then concentrated to 10 mL. Analysis for MP in feces and tissues was evaluated by flame photometric gas chromotography.

Mortality: Mortality data were collected from the excretion-retention dosing of each study. Additional mortality dosing was performed for each combination to insure 3 replicates of 12 each. The possible potentiation of MP by T was further studied. Mice received one-half LD_{50} doses (56T and 16MP mg/kg) and a 2:1 combination based upon the MP one-half LD_{50} (32T and 16MP mg/kg). The groups were: T (56 mg/kg), T (32 mg/kg), T (56) + MP (16 mg/kg), T (32) + MP (16 mg/kg), MP (16 mg/kg), and corn oil-dosed controls. Each group consisted of 4 replicates of 12. Mortality counts were made up to 48 h.

RESULTS AND DISCUSSION

Excretion: The excretion of radioactivity derived from ^{36}Cl-T, represented as percent of the administered dose, is reported in Table 1. During the 8 days, percentages recovered in the feces and urine from T alone were similar to previous reports (CROWDER and DINDAL 1974, CHANDURKAR and MATSUMURA 1979a). Excretion of

TABLE 1

Recovery of ^{36}Cl from urine and feces of male mice orally dosed with ^{36}Cl-toxaphene. Average % administered dose through 8 days.

	TREATMENT[a]			
	T[b]	T-C	T-MP	T-MP-C
Urine				
Initial Dose	18.2a	25.0a	21.2a	17.5a
Redose	28.0a	17.4a	23.4a	25.9a
Feces				
Initial Dose	42.5a	54.7a	31.5b	27.5b
Redose	58.8a	47.0a	46.8a	57.2a

[a]Means followed by the same letter within each dose are not significantly different at the 0.05 level.

[b]T-Toxaphene, 25 mg/kg; MP-methyl parathion, 12.5 mg/kg, C-chlordimeform, 3.2 mg/kg.

^{36}Cl in urine was not altered by combinations of C and/or MP at either dose. Following the initial dose less ^{36}Cl was excreted in the feces when combined with MP or C-MP. Since C did not alter the pattern, it is assumed that MP was responsible for this alternation. Following the redose, reductions were noted with C and MP, but they were not statistically significant.

Excretion of ^{14}C following a single oral dose of ^{14}C-C was not affected by combinations of T and/or MP (Table 2). Although differences between some values were noted, the large variance resulted in these differences being non-significant. In all cases with the initial dose, at least 83% of the ^{14}C-chlordimeform equivalents were eliminated in the excretion during 8 days. This finding agrees with KNOWLES and BENEZET (1977) where 95.5% of the administered dose was eliminated in mouse urine and feces by 96 h. Following a second dose, total excretion of ^{14}C in C alone and C-T was reduced approximately 20-25%; excretion via feces apparently accounted for this change.

The excretion of PNP, a metabolite of MP, was decreased by the addition of T. The mean total amount of PNP sampled from urine 96 h after dosing was 172.5 µg from MP-mice, and 67.5 µg from those treated with T-MP. No MP or methyl paraoxon were found in the collective feces extractions. An unidentified peak which eluted

TABLE 2

Recovery of ^{14}C from urine and feces of male mice orally dosed with ^{14}C-chlordimeform. Average % administered dose through 8 days.

	TREATMENT[a]			
	C[b]	C-T	C-MP	C-T-MP
Urine				
Initial Dose	31.9a	28.1a	38.2a	30.7a
Redose	37.2a	26.4a	19.8b	23.1a
Feces				
Initial Dose	75.0a	77.5a	75.6a	53.2a
Redose	49.7a	50.0a	43.0a	66.2a

[a]Means followed by the same letter within each dose are not significantly different at the 0.05 level.

[b]C-chlordimeform, 3.2 mg/kg; MP-methyl parathion, 12.5 mg/kg, T-toxaphene, 25 mg/kg.

prior to the parent and oxon peaks was the only compound observed. However, there was no difference in the mean peak height of this compound between MP and T-MP samples.

Tissue Retention: Retention of ^{36}Cl in several tissues of mice is reported in Table 3. Lipid was the only tissue which displayed no significant changes as a result of combining T with C and/or MP. Adding C to T led to more ^{36}Cl being deposited in the brain, muscle and testes. The T-MP combination resulted in more ^{36}Cl in brain, but less in kidney and liver. When both C and MP were added to T the results were similar to T-MP; brain had significantly more, and kidney and liver had less ^{36}Cl than T alone.

In general the amount of ^{36}Cl retained by tissues increased after a redose. However, tissues from mice sacrificed 33 days after the redose had ^{36}Cl levels approaching those of single dosed mice. Following a redose, ^{36}Cl concentrations were significantly elevated in kidney by T-C and T-MP-C combinations and higher in muscle by T-MP and T-C, yet were significantly lower in lipid from T-C and T-MP-C (Table 3). Thirty three days after the redose, ^{36}Cl levels in lipid remained significantly reduced in T-C dosed mice while T-MP and T-MP-C led to higher levels of retention in the liver.

Retention of ^{14}C from radiolabelled C alone was low in the tissues of mice which were examined (Table 4). This is similar to the report for white rats (KNOWLES and SEN GUPTA 1970). Combining T and/or MP with C resulted in lowering ^{14}C deposition in some tissues. Addition of MP led to a drop of ^{14}C levels in the liver, while MP, T, and both together when added to C caused less ^{14}C to be retained in lipid, muscle, and the testes.

TABLE 3

Retention of ^{36}Cl in ppm by tissues of male mice following doses of ^{36}Cl toxaphene.

Treatment	Tissue[a]					
	Brain	Kidney	Lipid	Liver	Muscle	Testes
Initial Dose						
T	0.2a	0.7a	10.6a	0.5a	1.2a	0.8a
T-C	1.8b	1.2a	11.7a	0.9a	3.8b	4.2b
T-MP	0.5b	0.4b	9.9a	0.3b	0.5a	0.7a
T-MP-C	0.7b	0.3b	10.9a	0.4b	0.5a	0.5a
Redose - 8[b]						
T[c]	2.6a	0.3a	17.0a	0.7a	2.0a	2.6a
T-C	2.1a	1.4b	7.9b	2.1a	2.8b	2.3a
T-MP	3.2a	0.5a	16.7a	1.9a	3.7b	2.8a
T-MP-C	2.2a	1.2b	2.8b	0.6a	2.0a	2.7a
Redose - 33[b]						
T	1.8a	0.5a	3.6a	0.4a	1.5a	2.2a
T-C	1.2a	0.7a	2.0b	0.3a	1.5a	1.6a
T-MP	1.4a	0.4a	3.2a	1.0b	1.4a	2.4a
T-MP-C	2.5a	0.8a	3.2a	1.1b	2.5a	2.4a

[a]Means followed by the same letter within each tissue for a given dose are not significantly different at the 0.05 level.

[b]Days after receiving redose.

[c]T-toxaphene, 25 mg/kg; MP-methyl parathion, 12.5 mg/kg, C-chlordimeform 3.2 mg/kg.

TABLE 4

Retention of ^{14}C in ppm by tissues of male mice orally dosed with ^{14}C-chlordimeform.

Treatment	Tissue[a] Brain	Kidney	Lipid	Liver	Muscle	Testes
Initial Dose						
C[b]	0.6a	0.8a	0.3a	0.6a	0.4a	0.6a
C-T	0.4a	0.8a	0.1b	0.5a	0.2b	0.3b
C-MP	0.4a	0.5a	0.1b	0.3b	0.2b	0.2b
C-T-MP	0.5a	0.7a	0.1b	0.4a	0.2b	0.2b
Redose						
C	0.2a	0.4a	0.2a	0.7a	0.2a	0.1a
C-T	0.5a	0.3a	0.1a	0.6a	0.1a	0.2b
C-MP	0.4a	0.6a	0.3a	1.0b	0.3a	0.4b
C-T-MP	0.4a	0.5a	0.4a	1.2b	0.3a	0.4b

[a]Means followed by the same letter within each tissue are not significantly different at the 0.05 level.

[b]C-chlordimeform, 3.2 mg/kg; MP-methyl parathion, 12.5 mg/kg; T-toxaphene, 25 mg/kg.

Following the redose, C-T led to only one significant change by increasing the level of ^{14}C in the testes. C-MP also increased ^{14}C levels in testes as well as in the liver. When all 3 insecticides were combined, significant increases in ^{14}C levels occurred in the testes, liver, and lipid.

Mortality: In the excretion-retention studies, mortality occurred only in treatments containing MP, i.e., MP, T-MP, MP-C, and T-MP-C (Table 5). All mortality occurred within 3 h of dosing. It appeared that MP toxicity was slightly enhanced by C and slightly lowered by T, but none of these differences were significant. The mortality study using only MP and T verified the above in that no potentiation of MP was produced by T.

TABLE 5

Mortality of mice from combinations of toxaphene (T), methyl parathion (MP), and chlordimeform (C).

Treatment	Number of Replicates (12/replicate)	$\bar{X}$ Mortality (%)[a]
T, MP study:		
T (32)[b]	4	0 a
T (56)	4	0 a
MP (16)	4	34.1 b
T (32) - MP (16)	4	20.8 b
T (56) - MP (16)	4	29.2 b
Excretion-Retention Studies:		
T	3	0 a
T-MP	3	20.3 b
T-MP-C	3	29.2 b
T-C	3	0 a
MP	5	25.5 b
MP-C	3	29.2 b
C	3	0 a

[a]Means followed by the same letter are not significantly different at the 0.05 level.

[b](mg/kg)

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