Stability of [^{14}C]Lindane, [^{14}C]Chlorpyrifos, and Coumaphos in Model Cattle Dip

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Stabilities of lindane, chlorpyrifos, and coumaphos were studied in simulated cattle dipping vat. The half-life (DT_{50}) of lindane was found to be only 4 days. By 9 weeks' time, 99% of the lindane had dissipated from model vat. Chlorpyrifos was insoluble in water. The first-phase half-life of chlorpyrifos was found to be 22 days. Bound chlorpyrifos in soil was found at less than 5%. Stability of coumaphos was found to be related with the pH of water. The first-phase half-life (DT_{50}) of coumaphos in the control vat was 99 days, which increased to 114 days in the vat containing superphosphate fertilizer used as buffer but remained 98 days with sodium citrate. Chlorferon, potasan, and an unidentified compound were found to be present with coumaphos.

Keywords: *Acaricides; lindane; chlorpyrifos; coumaphos; cattle dip*

INTRODUCTION

Ectoparasites like ticks and mites are directly pathogenic as well as transmitters of many pathogenic organisms. The organochlorine and organophosphate acaricides represent major classes of pesticides that are widely used to control ectoparasites on livestock. Among the organochlorine pesticides, lindane (γ-benzene hexachloride) is one of the most effective acaricides used in many countries of the world. Organophosphates such as chloropyrifos [*O*,*O*-diethyl *O*-(3,5,6-trichloro-2-pyridinyl)phosphorothioate] and coumaphos [*O*,*O*-diethyl *O*-(3-chloro-4-methyl-2-oxo-2*H*-1-benzopyran-7-yl] are also highly effective, and the latter is used (Kearney et al., 1986) especially for control of *Boophilus microplus* (canestrini) and *Boophilus annulatus* (say), the southern cattle ticks. Spraying, dust bags, animal body washing, and acaricide dips are currently practiced methods to control ticks in many countries. Among these, the cattle dip is a popular practice in many parts of the wold because it provides thorough treatment of the herd and the same acaricide solution can be used repeatedly. Besides, the dip system contributes to better disposal of acaricidal residues. However, the use of these chemicals at the right concentration in the cattle vats is very important for effective control of ticks and mites. Low strength of the acaricides in cattle dips has been identified as one of the factors for development of tick resistance to acaricides (Hassan, 1994). So, to improve the stability of the cattle dips, it is important to know the path of acaricide degradation in the cattle dips. Nuclear techniques using the labeled pesticide(s) are important tools to monitor the fate and to identify trace levels of pesticide for this type of study.

The aim of this work was to evaluate the stability of [^{14}C]lindane, [^{14}C]chlorpyrifos, and coumaphos in model cattle dips in current practices and to develop procedures to improve the stability of coumaphos by using calcium superphosphate fertilizer and sodium citrate to change the pH in the model vat.

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MATERIALS AND METHODS

Chemicals. [^{14}C]Lindane (with specific activity 17.5 mCi/mmol), [^{14}C]-chlorpyrifos (9.1 mCi/mmol), and analytical standards of lindane and cholorpyrifos were received from the International Atomic Energy Agency (IAEA). Coumaphos and its metabolites potasan and chlorferon were supplied by from Bayer AG, Germany. Radiochemical purities were determined by TLC and found to be 97% for lindane and 96.5% for chlorpyrifos.

Methods. *Model Dips, Sampling, and Analysis.* In order to simulate the field practices, model vats were made using rod−cement−concrete, each with a 50 L capacity of water. Stock solutions of [^{14}C]lindane and [^{14}C]chlorpyrifos were prepared separately in 10 mL of acetone. The cold formulation masses of the radioactive compounds were calculated on the basis that the end concentration in the model vat of each 40 L of water would be 100 mg/mL. To prepare a ^{14}C-homogeneous solution, the cold formulation and radiolabeled compound in acetone were mixed in a volumetric flask. Acetone, 50 mL, was further added and stirred for 10 min by a vortex mixer. The final mixed solution was quantitatively transferred in each 40 L vat containing tap water. The same water level was maintained throughout the experimental period. Soil collected from the vicinity of cattle yard was added to the vat as 1% of water every month. The model dips were kept in an environment similar to that of the actual field vat. Acaricides in the model dips were homogenized with a stick before each sampling.

Duplicate samples (10 mL) of the suspension were taken from each vat at each sampling time, and 0.5 mL aliquots were placed in vials and analyzed using a liquid scintillation counter (LSC) (Packard Tri-Carb 1000) for counting radioactivity in the suspension; the samples were mixed with 10 mL of scintillation fluid. All samples were quench corrected for background radioactivity. The remaining suspension was centrifuged at 2000 rpm for 15 min, and 0.5 mL aliquots of the supernatant were also counted for radioactivity. About 1 g of soil sample was collected by filtering the suspension of vat containing [^{14}C]lindane and [^{14}C]chlorpyrifos and was extracted with methanol using Soxhlet extraction for 4 h. The extract obtained was concentrated, and aliquots of 1 mL were counted to determine the quantities of lindane and chlorpyrifos, respectively. The activity that remained in the soil after extraction was determined by combustion in a biological oxidizer (Harvey 0X-600) and then by liquid scintillation counting. The activity in soils represents the non-extractable (bound) pesticides.

The stability of coumaphos (Asuntol containing 50% a.i., Bayer, Leverkusen, Germany) was studied in three separate

S0021-8561(96)00796-0 CCC: $14.00

Table 1. Persistence of Radioactive Lindane in Model Vat

time after application	pH	concentration (mg/mL) in suspension	concentration (mg/mL) in supernatant
0 day	6.5 (before adding soil)	77.7	35.5
2 days	8.4	55.5	22.0
1 week	8.7	31.9	13.9
3 weeks	9.0	2.7	3.4
5 weeks	9.0	1.1	1.4
7 weeks	9.5	0.6	0.7
9 weeks	9.3	0.3	0.4

Table 2. Extractable and Non-Extractable (Bound) Lindane in Soil (Sediment) of Model Vat

time after application	quantity of lindane in soil (μg/g) extractable	bound	% bound
0 day	64.1	11.2	14.9
3 weeks	2.5	2.5	50.0
6 weeks	1.0	1.5	60.0
9 weeks	0.5	0.7	58.3

model vats at different pH, and the concentration of the solution was 200 mg/mL. To decrease or increase the pH of the solution, 1% superphosphate fertilizer or sodium citrate was added to the water (40 L) of the model vat, respectively. In addition, a vat with a normal pH was marked as the control. Samples of 100 mL from each vat were taken in duplicate for extraction with ethyl acetate. A HPLC method was used for the analysis of coumaphos and its degradation products. The HPLC (Waters 486) was fitted with a UV detector at 254 nm, using a 3.9 × 150 mm C_{18} reverse phase column. The solvent system used was acetonitrile:water (65:35). Identifications of HPLC peaks were made by comparing the retention times with those of the analytical standards of coumaphos, chlorferon, and potasan. Simultaneous confirmation of the compounds was made by thin-layer chromatography (TLC). TLC was done with silica gel 60 GF_{254}. TLC plates were developed in two different ratios of benzene−ethyl acetate (9:1 and 7:3), with visualization under UV light.

RESULTS AND DISCUSSION

The persistence of radioactive lindane in suspension and in supernatant water in the model vats was monitored during 9 weeks, and the observations are shown in Table 1. The pH on day 0 was 6.4 (before adding soil), which increased to 8.4 on the 2nd day and reached its highest value at 9.5 by 7 weeks' time. The concentration of lindane on day 0, i.e., immediately after application, was 77.7 mg/mL in suspension and 33.5 mg/mL in supernatant. By 1 week, about 60% of the radioactive material had dissipated in both cases. After that, the dissipation rate was found to be faster in suspension. By 5 weeks' time about 99% was dissipated from the suspension. In case of supernatant, 99% dissipation was found by 9 weeks. Extractable and non-extractable (bound) lindane in soil (sediment taken from the model vat) are presented in Table 2. At day 0, about 15% of radioactive lindane was seen to be non-extractable from soil, and by 3 weeks it was 50%. Formation of bound residues increased with progress of time, and it is reported (Raghu et al., 1986) to be related to the neutral and alkaline soils. The pH in the model vat was increased from 6.4 to 9.5 and it probably could help the mineralization of pesticides. Most portions of lindane, about 95% of the initial concentration, were found to be dissipated from the model vats by 3 weeks time, as shown in both Tables 1 and 2. It indicates that most of the radioactive lindane is dissipated by volatilization from the suspension and the soil of the model vat. The first-phase half-life (DT_{50}) of lindane was found to be only 4 days. The DT_{50} of lindane has been mentioned (Worthing et al., 1991) to be 191 days at pH 7 and 11 h at pH 9. When lindane undergoes environmental degradation under humid or submerged conditions and in field conditions, its half-life varies from a few days to 3 years, depending on type of soil, climate, depth of application, and other factors (Herbst et al., 1991).

Table 3. Persistence of Radioactive Chlorpyrifos in Model Vat

time after application	pH	concentration (mg/mL) in suspension	concentration (mg/mL) in supernatant
0 day	6.5 (before adding soil)	87.4	8.5
2 days	8.5	76.4	7.8
1 week	8.7	44.3	8.1
3 weeks	9.0	42.4	8.6
5 weeks	9.3	29.9	9.9
7 weeks	9.1	30.0	9.4
9 weeks	9.1	32.2	9.8

Table 4. Extractable and Non-Extractable (Bound) Chlorpyrifos in Soil (Sediment) of Model Vat

time after application	quantity of chlorpyrifos in soil (μg/g) extractable	bound	% bound
0 day	323.0	15.4	4.5
3 weeks	191.6	7.8	3.9
6 weeks	84.8	3.8	4.3
9 weeks	79.9	2.4	3.0

In Table 3, persistence of radioactive chlorpyrifos in suspension and in supernatant water is presented. In this case, too, pH increased sharply from 6.5 to 8.5 and remained around 9.0 throughout the experimental period. On day 0, chlorpyrifos was found to be 87.4 mg/mL in suspension and 8.5 mg/mL in supernatant. The first-phase half-life of chlorpyrifos was found to be 22 days. It has been reported (Worthing et al., 1991) that the stability of chlorpyrifos and the rate of hydrolysis increase with pH; DT_{50} varies from 1.5 to 100 days depending on pH and temperature of the solution. The solubility of chlorpyrifos in water at 25 °C has been mentioned (Worthing et al., 1991) to be 2 mg/L. From this experiment (Table 3), it is observed that by 3 weeks' time about 50% of the initial concentration in suspension was dissipated while by that time it was almost unchanged in supernatant. After 3 weeks, the concentration in suspension found to be unchanged but in the supernatant it increased to 115% of the initial concentration. Table 4 represents the extractable and non-extractable (bound) chlorpyrifos from soil. From these tables, it is indicated that chlorpyrifos was almost insoluble in vat water and the dissipation was found mostly from the soil. The higher concentration found in supernatant water with progress of time may be due to mobilization of chlorpyrifos from soil to water either by dissolution or emulsification. It has been reported (Scheunert, 1992) that in the complex situation in soil with pesticides adsorbed in part at soil particles and in part in soil aqueous phase, more reaction pathways are possible, resulting in photolytic reactions of those pesticides which normally are not able to react. The percent of bound chlorpyrifos in soil of the vat was found to be less than 5% throughout the experimental period.

Degradation of coumaphos in the model vat at different pH was studied using superphosphate fertilizer to decrease pH and sodium citrate to increase pH. The results are presented in Table 5. The pH of tap water used was neutral, but when the tap water was kept in the model vat, its pH increased to 8.5. The increase in pH is believed to be due to the interaction of water with

Table 5. Degradation of Coumaphos in Model Vat at Various pH[a]

time	control		+ superphosphate		+ sodium citrate	
(days)	pH	concentration	pH	concentration	pH	concentration
0	8.5	204.2 ± 2.6	6.4	209.5 ± 18.3	8.9	189.00 ± 7.6
15	8.5	197.7 ± 0.0	6.5	217.2 ± 10.8	8.8	183.9 ± 7.1
30	8.6	184.1 ± 9.8	6.8	195.8 ± 0.2	8.8	183.6 ± 16.9
60	8.5	126.5 ± 0.7	7.0	133.3 ± 2.6	8.9	115.0 ± 1.3
90	8.5	101.0 ± 1.0	8.0	117.1 ± 2.8	9.1	89.7 ± 33.2
120	8.5	88.6 ± 1.9	8.3	101.2 ± 8.4	9.0	81.0 ± 19.7
150	8.4	93.0 ± 4.2	8.4	94.1 ± 1.4	9.0	89.9 ± 7.2
180	8.6	92.8 ± 4.7	8.2	95.9 ± 3.9	9.2	88.7 ± 5.5

[a] Concentrations are presented as the mean ± standard deviation of two replicates ($x \pm$ sd), in mg/mL.

cement−concrete wall (alkaline) of the vat. At the beginning of the experiment, due to addition of superphosphate fertilizer at a rate of 1% of the water content, the pH in the vat decreased to 6.4 which with progress of time gradually increased to 8.0 by 90 days. This increase may be due either to gradual inactivation of superphosphate fertilizer or the long standing time of the water in the vat. To keep the pH of water constant, repeated addition of superphosphate may be practiced but it could bring a bulk to the water and additional cost might be required. On the other hand, in the vat containing sodium citrate, the pH of water increased about 9.0 and it remained almost 9.0 throughout the experimental period. The first-phase half-life of coumaphos in control vat was 99 days, which increased to 114 days due to addition of superphosphate fertilizer but remained at 98 days in vat with sodium citrate. Very little difference in the dissipation rate was found in all of the model vats without charging with buffers, and the rate of dissipation shows the same trend during the 180 days of experiment. Waggoner (1985) reported the half-life of coumaphos in sandy loam soil under laboratory condition to be about 300 days.

Degradation of coumaphos occurs anaerobically and aerobically, and in aged vats coumaphos degrades by reductive dechlorination under anaerobic conditions to potasan (Karns et al., 1995). It was reported (Shelton and Karns, 1988) that degradation of coumaphos is inhibited due to increase in pH.

Tanu Jindal et al. (1996) conducted experiments with coumaphos at different pH. They found that dissipation of pesticide was rapid at pH 7.0 and that, by reducing the pH to 5.0, the degradation of organophosphate acaricide can be minimized. It is believed that degradation of pesticide can be further prevented by addition of bactericides, viz. $CuSO_4$, $MgCl_2$, etc., but they also reported that very little difference was found by addition of bactericides with buffer to prevent acaricide degradation. Chlorferon available in the model vats is presented in Table 6 with progress of time. Initially, on day 0 of the experiment chlorferon was 7−8% of the coumaphos content. Chlorferon content decreased gradually until 90 days of the experiment, and then its contents in all the vats were more or less constant. Potasan available in the vats is presented in Table 7. Its content was nearly 2% of the coumaphos content on day 0 and decreased with time. At the later part of the experimental period potasan could not be detected in any of the model vats. The potasan byproduct is produced from the anaerobic dechlorination of coumaphos, and this byproduct is toxic to cattle (Shelton and Karns, 1988). In the United States, when the potasan level exceeds 300 mg/L, the dip vat must be taken out of service and the liquid disposed in a holding pond

Table 6. Concentrations of Chloropheron Available in Model Vat Having Asuntol (Coumaphos) with Progress of Time[a]

time (days)	control	+ superphosphate	+ sodium citrate
0	16.5 ± 2.6	14.0 ± 1.8	13.4 ± 0.2
15	14.2 ± 0.5	16.1 ± 0.6	14.2 ± 0.5
30	15.3 ± 0.4	16.9 ± 2.2	18.2 ± 0.8
60	14.2 ± 1.0	10.9 ± 0.5	12.8 ± 0.6
90	8.8 ± 0.0	8.5 ± 0.0	11.9 ± 1.0
120	8.8 ± 0.0	9.2 ± 0.5	9.8 ± 0.0
150	8.7 ± 0.1	8.1 ± 0.4	8.9 ± 0.2
180	7.1 ± 2.1	8.2 ± 0.5	8.1 ± 0.8

[a] Concentrations are presented as mean ± standard deviation of two replicates ($x \pm$ sd), in mg/mL.

Table 7. Concentrations of Potasan Available in Model Vat Having Asuntol (Coumaphos) with Progress of Time[a]

time (days)	control	+ superphosphate	+ sodium citrate
0	4.1 ± 0.0		3.8 ± 0.2
15	3.1 ± 0.3	3.9 ± 0.0	3.5 ± 0.3
30	3.2 ± 0.2	3.8 ± 0.1	4.2 ± 0.2
60			2.7 ± 0.0
90		2.2 ± 0.0	
120			
150			
180			

[a] Concentrations are presented as mean ± standard deviation of two replicates ($x \pm$ sd), in mg/mL.

Table 8. R_f Values of Extracted Sample and Different Standards in Benzene:Ethyl Acetate as Solvent System

	solvent ratio	
sample	9:1 v/v	7:3 v/v
chlorferon	0.44	0.63
potasan	0.74	0.80
coumaphos		0.89
asuntol (coumaphos)		0.90
mixed standard		
chlorferon	0.44	0.65
potasan	0.78	0.83
coumaphos	0.90	0.87
extracted sample		
chlorferon	0.44	0.62
potasan	nd[a]	nd
coumaphos	0.88	0.87
unidentified	0.96	0.93

[a] nd, not detected.

(Grice et al., 1996). It was also observed that the enzyme parathion hydrolase was capable of degrading potasan into 4-methylbelliferon and that under dip vat conditions the rate of potasan hydrolysis was considerably greater (Copella et al., 1990).

R_f values of the extracted samples along with different analytical standards are presented in Table 8. In extracted samples nothing was found corresponding to potasan, but in both solvent ratios, an unidentified compound with R_f values of 0.96 and 0.93 was found.

CONCLUSION

From the study it can be concluded that lindane dissipates from the model animal dipping vat quite rapidly, mostly due to volatilization, with a half-life of only 4 days. Chlorpyrifos remained mostly undissolved in water, and its first-phase half-life was 22 days. In model vats, pH increased with progress of time. Degradation of coumaphos was related to pH changes in the vat. The first-phase half-life of coumaphos was 98−114 days, and then the compound remained stable for the next 3 months.

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Received October 21, 1996. Revised manuscript received February 10, 1997. Accepted March 31, 1997.[⊗] Financial support received by the International Atomic Energy Agency as Research Contract 7109/RB is gratefully acknowledged.

JF960796E

[⊗] Abstract published in *Advance ACS Abstracts*, June 15, 1997.