Improved Extraction of Chlorinated Hydrocarbon Pesticides from Animal Tissues

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Several researchers have suggested that DDT and dieldrin are bound to animal tissues (1, 2, 3, 4). If binding of chlorinated hydrocarbon pesticides to tissues is significant, their extraction with petroleum ether might be facilitated by the addition of surface active agents. Surfactants should have a dispersive effect on fatty tissues and might disrupt hydrophobic bonding between pesticide and tissue components. Consequently, various surfactants were included in the extraction procedure developed by Mills (5) and recommended by the Federal Food and Drug Administration (6) to determine if these materials could increase the extractability of the chlorinated hydrocarbon pesticides.

Materials and Methods

Omental fat collected from dairy cows fed dieldrin, and a mixture of lindane, heptachlor and DDT, was analyzed in the presence and absence of surface active materials. Five gram portions of omental fat were ground with anhydrous sodium sulfate and either 5 g of Span 80 (sorbitan monooleate), Span 60 (sorbitan monostearate), crude soybean lecithin (UF-1, A. E. Staley, Decatur, Illinois) or Triton X-100 and extracted with petroleum ether. Control samples were ground in the absence of surfactant

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and extracted with either petroleum ether (5) or 2:1 chloroform: methanol (7). Chloroform-methanol was used as a solvent to ascertain if this polar-nonpolar mixture would extract a greater concentration of the pesticides.

Since beef fat was incompletely soluble in a total of 15 ml of petroleum ether, the recommended procedure of Mills (5) was modified to use 30 ml of petroleum ether for the initial transfer of fat for cleanup followed by two 15 ml rinses. This solution that was transferred in petroleum ether was partitioned against 30 ml of acetonitrile saturated with petroleum ether. Additional partitioning and subsequent cleanup were according to the procedure of Mills (5). An F and M model 810 gas chromatograph equipped with an electron capture detector and a 6 mm X 1.23 m glass column packed with 4% SE-30 on 80 to 90 mesh Anakrom ABS was used to separate and quantify the pesticides.

Results and Discussion

The data in Table 1 are the averages of duplicate trials and include the variation between samples. The efficacy of added Span 80 in removing chlorinated pesticides is apparent, particularly in the case of dieldrin and heptachlor epoxide. The data for the trials which involved Span 60 are not shown since these values were less than those for the control samples. However, the difference in performance between Span 60 and Span 80 is striking, in view of their structural similarity and their close HLB (hydrophile-lipophile balance) values (8). Part of the improved performance of Span 80 may be attributed to its liquid

TABLE 1

The efficacy of added surfactants on the extraction of chlorinated hydrocarbon pesticides from omental fat.

Surfactant	Concentrations of pesticides extracted, ppm[a]			
	Dieldrin	Lindane	Heptachlor epoxide	DDT[b]
Control (mills procedure)	0.007 ± .001	0.018 ± .002	0.039 ± .001	0.208 ± .004
Control (2:1 chloroform:methanol)	---	0.045 ± .003	0.030 ± .002	0.345 ± .015
Span 80	0.690 ± .020	0.045 ± .004	0.089 ± .005	0.370 ± .080
Crude lecithin	0.013 ± .002	0.038 ± .007	0.026 ± .001	0.345 ± .025
Triton X-100	---	0.022 ± .003	0.026 ± .001	0.199 ± .013

[a] µg/g tissue

[b] Represents the sum of the o,p' and p,p' peaks

nature at room temperature, which apparently facilitates admixture with the tissues. Crude lecithin was less effective than Span 80, whereas Triton X-100 was essentially ineffective. Although these preparations are liquid at room temperature, they are structurally unrelated to Span 80. Analyses of petroleum ether extracts of 5 g samples of Span 80 and crude lecithin under the above conditions indicated the absence of detectable pesticides.

Some difficulty was encountered in transferring the fatty material extracted with chloroform-methanol because of the presence of phospholipids that were insoluble in petroleum ether. This, in part, could explain some of the low recoveries of pesticides, particularly dieldrin and heptachlor epoxide. Binding of chlorinated hydrocarbons to the phospholipids is possible, and quantitative recovery might be difficult.

The variation between concentrations of pesticides extracted is within the limits of experimental error except for the samples containing DDT that were extracted in the presence of Span 80 and crude lecithin. Regardless of the fact that the variability is great, the evidence that both of these surfactants improve the extractability of chlorinated hydrocarbons is not altered.

A preliminary investigation showed no improvement in the efficiency of fat extraction in the presence of Span 80 and crude lecithin when compared to the amount of fat extracted from control samples that contained no added surfactant. This would suggest that the surfactant is disrupting hydrophobic bonding

between the fatty tissue and the pesticide.

These data indicate that the extraction of chlorinated pesticides by petroleum ether from fatty tissues at the levels studied in this report is facilitated by the addition of surfactants such as Span 80.

Summary

An improved procedure is presented whereby greater concentrations of chlorinated hydrocarbon pesticides are removed from fatty animal tissue. This procedure employs surfactants such as Span 80 and crude lecithin in the presence of petroleum ether to extract the fat and pesticide.

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