Starch–Xanthate-Encapsulated Pesticides: A Preliminary Toxicological Evaluation

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The relative safety of starch–xanthate-encapsulated parathion compared to parathion adsorbed on attapulgus clay granules (20–30 mesh) was studied. In vitro methods were employed to determine the relative solubility, volatility, and cutaneous permeability of encapsulated and adsorbed [35S]parathion. Compared to the adsorbed formulation, starch–xanthate capsules less readily released [35S]parathion into aqueous solvents such as perspiration, less readily released [35S]parathion to air (dry or wetted), and reduced cutaneous penetration and accumulation of [35S]parathion. In a flowing system the leaching rate of [35S]parathion from starch–xanthate capsules was directly related to temperature, H^+ concentration, and the flow rate of the aqueous solvent and inversely related to time. Wetting of the encapsulated or adsorbed formulation significantly increased the volatilization of [35S]parathion. Reduced solubility, volatility, permeability, and accumulation of [35S]parathion indicate that the starch–xanthate formulation is an improved granular formulation from the standpoint of pesticide handler safety.

From a toxicological perspective the ideal pesticide or herbicide formulation is that formulation which is most effective against the target organism and poses the least risk to nontarget organisms, especially man. There is a continuing need for the development of innovative pesticide formulations which maximize activity and minimize adverse effects (Hedin, 1982). For pesticide workers the greatest exposure risk occurs during the handling, mixing, and filling of mixing tanks and application equipment, and the main route of exposure is percutaneous (Coutts, 1980). One of the least hazardous formulations is the granular type (Speight, 1980). Recently introduced controlled-release formulations offer a very effective means for reducing the risks from highly volatile pesticides (Speight, 1980) and, in some cases such as starch–xanthate capsules (Shasha, 1980), may reduce the risks of percutaneous absorption. Recent studies indicate that starch-encapsulated pesticides are equally effective, relative to unencapsulated pesticides, with regards to the target pest (Otey, 1981) and also offer economic advantages because of controlled release (Shasha et al., 1981).

The purpose of this study was to gather data for a preliminary assessment of the relative safety of starch–xanthate-encapsulated pesticides compared to pesticides adsorbed on attapulgus clay granules. In vitro methods were employed to determine the relative solubility, volatility, and cutaneous permeability of encapsulated and adsorbed formulations. It was reasoned that (1) reduced solubility in aqueous salines would lessen the risk of accumulation via contact of granules or fine granular dusts with the buccal epithelia, nasal epithelia, eyes, etc., (2) reduced volatility would lessen risk of inhalation, and (3) reduced cutaneous permeability would lessen the risk of accumulation via skin contact.

MATERIALS AND METHODS

Chemicals. All analytical chemicals were reagent grade. Solvents used in extraction procedures and thin-layer solvent systems and water used in preparation of reagents were distilled in glass. [35S]Parathion (15.4 mCi/mmol) was custom synthesized by Amersham Corporation (Arlington Heights, IL). The radiochemical purity was determined by thin-layer chromatography on 5 × 20 cm glass plates coated 0.25 mm thick with silica gel G F-254

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(Brinkman) by using trimethylpentane–acetone–chloroform (70:25:5 v/v) as the developing solvent system (Getz, 1971). Parathion was visualized at 254 nm. Radioactive contaminants were identified by scanning the TLC plates on a Searle Actigraph III radiochromatogram scanner. Radioactivity in the contaminants and parathion was quantified by scraping 1 cm wide bands over the length of the developed plate and counting each band in 10 mL of Hydrofluor (National Diagnostics, Sommerville, NJ) with a Packard Tri-carb 460 CD by using standard liquid scintillation counting (LSC) procedures and corrections for the 35S decay rate. A reference standard of parathion (Chem Service, Westchester, PA) was spotted along with the [35S]parathion. Two radiolabeled contaminants containing a total of 5% of the original radioactivity were detected, and 95% of the activity was recovered in parathion. Thin-layered chromatograms developed in hexane–dichloromethane–methanol (70:20:10 v/v) did not reveal any radiochemical contaminants. The chemical purity of [35S]parathion was determined by gas chromatography on a temperature-programmed (150–250 °C, 8 °C/min) Tracor Model 222 with a flame photometric detector (190 °C) and a 4 mm × 1.82 m column packed with 10% OV-101 on 80–100-mesh Chromosorb W-HP; the carrier gas was N_2, 65 mL/min. The retention time (33.7 min) of the [35S]parathion was the same as that of the reference standard; no contaminants were detected.

Test Agents. Encapsulated [35S]parathion (SX), 13.5% active ingredient, was prepared by the starch–xanthate and ferric chloride chloride procedure (Shasha, 1980) at the Northern Regional Research Center, USDA (Peoria, IL). The adsorptive formulation (AF), 10.5% active ingredient, was prepared as follows: 100 mg of stock [35S]parathion was dissolved in 0.5 mL of methanol and small portions were mixed with 0.6 g of attapulgus clay (14–30 mesh), the methanol being evaporated under N_2 after each addition. The attapulgus clay was obtained courtesy of Stauffer Chemical Co. (Mountain View, CA). Both formulations were sized to 20–30 mesh after preparation. The percent active ingredient was determined based on the reported specific activity after verification of the radiochemical and chemical purity of the encapsulated and adsorbed parathion. Radiochemical and chemical purity of parathion from SX and AF was determined after digesting a 5–10-mg sample in 1 mL of 1 N HCl overlayed with 1 mL of toluene in a tightly capped (Teflon liner) 4-dram vial at 55–60 °C for 32 h. The digest was cooled and made to one phase with isopropyl alcohol, and an aliquot was counted by LSC. Recovery of stock [35S]parathion added to starch capsules

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or attapulgus clay granules, and digested as described above, was 97.9% (SD = 1.2%, n = 3) and 97.8% (SD = 1.4%, n = 3), respectively. The isopropyl alcohol was transferred, with several washes, to a separatory funnel containing 5–10 mL of water and 5–10 mL of benzene. The digest was extracted 3 times with benzene, and the benzene extracts were pooled. On the basis of the radioactivity in the original digest, ^{35}S recovery from SX and AF in the benzene phase was over 97% and in the aqueous phase under 3%.

Test Systems. The leaching rate of [^{35}S]parathion from SX and AF was determined in a flowing system (Figure 1A). The elution column (MER chromatographic, Mountain View, CA) was a 5 cm × 0.9 cm Pyrex tube with 2 Kel-F end plugs and a 0.9-μm porous Teflon disk. SX or AF was weighed on the Teflon disk and then the column was filled with acid-cleaned glass beads (0.5 mm) and the column was assembled. The leachate was collected at predetermined intervals in culture tubes containing 1 mL of toluene to trap the parathion. The radioactivity in the leachate was determined by LSC after adding sufficient isopropyl alcohol to make the toluene and leachate to one phase. Distilled deionized water, 1 N HCl, and artificial perspiration (American Association of Textile Chemists and Colorists, 1979) were all tested as solvents for leaching [^{35}S]parathion from SX or AF.

The volatilization of [^{35}S]parathion from SX and AF was determined by using a simple aeration system (Figure 1B). The toluene traps were sampled at predetermined intervals, and radioactivity was determined by LSC in 10 mL of Hydrofluor.

The cutaneous permeability of [^{35}S]parathion from SX and AF was determined by using diffusion cells (Figure 1C). Epidermal sheets of pig skin were obtained by the method of Scheuplein and Blank (1973) from the abdominal area of suckling pigs (4–10 days old) and stored in sterile banking fluid at 4 °C until used (<2 weeks). Skin banking fluid consisted of 20% swine serum (Flow Laboratories, McLean, VA) in phosphate-buffered saline with 157 mg/L penicillin and 250 mg/L streptomycin. The cutaneous permeability experiment was repeated 3 times with six matched cells (three SX and three AF) and six 3.1-cm^2 epidermal disks cut from a single epidermal sheet for each experiment. Each epidermal disk was carefully inspected to ensure surface integrity. After the lower chamber (dermal side) of the diffusion cell was filled with sterile banking fluid, the skin disk was placed horizontally between the two half chambers with the stratum corneum facing up and dermal side down. SX of AF (5 mg) was added to the epidermal surface of the skin after assembly of the diffusion cell and then wetted with 20 μL of artificial perspiration. The assembly was sealed and placed on a multiple magnetic stirrer, samples of the receptor fluid were removed at predetermined intervals, and the radioactivity was determined by LSC. Fresh receptor fluid was replaced via the head pressure reservoir after each sample. At the end of each permeability experiment the diffusion cell was carefully disassembled, and the skin was removed, rinsed 5 times in water, and either counted intact in 10 mL of Hydrofluor by LSC or extracted with benzene and samples of the benzene extract and skin counted by LSC in Hydrofluor. Samples of the receptor fluid were also extracted with benzene. Benzene extracts of the skin and receptor fluid were spotted on TLC plates, developed, and scanned as previously described. The benzene extraction efficiencies for skin and receptor fluid (skin banking fluid) were 83% (SD = 13%, n = 6) and 53% (SD = 3%, n = 6), respectively. Low extraction efficiency of nonpolar

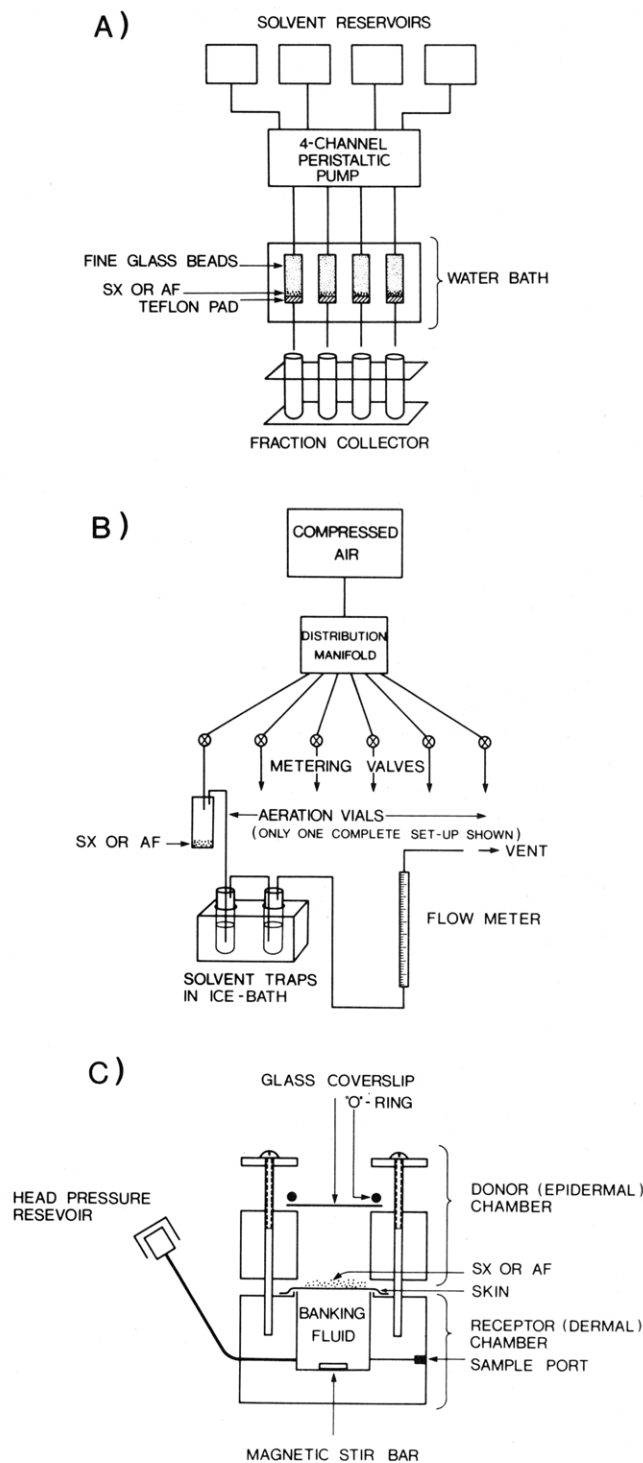**Figure 1.** (A) Elution system for determining solubility of encapsulated and adsorbed [^{35}S]parathion. Materials in contact with parathion were glass, Teflon, and Kel-F. (B) Aeration system for measuring relative volatility of encapsulated and adsorbed [^{35}S]parathion. Materials in contact with parathion were glass and Teflon. (C) Diffusion cell. Materials in contact with parathion were glass, Teflon, and stainless steel.

organic compounds from serum solutions is common. For example, the extraction efficiency of [G-^3H]benzo[a]-pyrene (17.4 Ci/mmol, Amersham Corp.) from phosphate-buffered saline (PBS) with three hexane extracts was 95.4% (SD = 0.7%, n = 3), from 5% serum in PBS 66.9% (SD = 1.4%, n = 3), and from 50% serum in PBS 48.2% (SD = 1.6%, n = 3).

Histology. Skin disks were treated for 24 h with 5 mg of SX or AF. An untreated control was run concurrently.

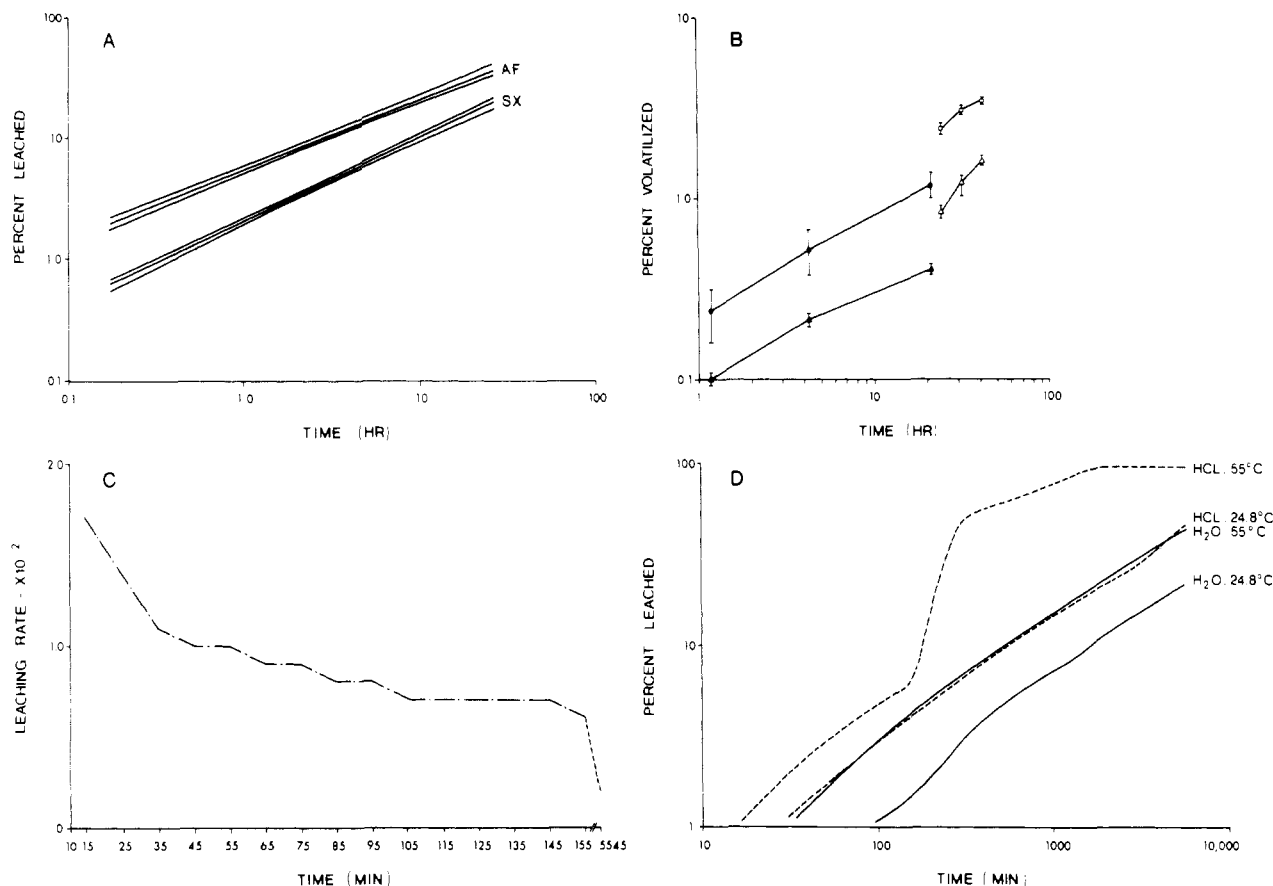**204** J. Agric. Food Chem., Vol. 31, No. 2, 1983

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Figure 2. (A) Leaching of parathion from starch–xanthate capsules (SX) and adsorptive formulation (AF). Results are expressed as the mean percent of total parathion originally added to elution columns. Upper and lower 95% confidence limits and the least-squares fit of the data are given for each treatment; n = 50 for each treatment. Columns were eluted with artificial perspiration at 37.5 °C. Flow rate was 0.27 mL/min. (B) Volatilization of parathion from starch–xanthate capsules (triangles) and adsorptive formulation (circles). Results expressed as percent (mean ± standard) deviation; n = 3) total parathion orginally added to aeration vials. Capsules and adsorptive formulation were wetted with artificial perspiration at hour 21 (open symbols). The air flow rate was 50 cm³/min at 20.1 °C. (C) Leaching rate of [35S]parathion from starch–xanthate capsules by using glass distilled deionized water at 24.8 °C. Results are expressed as the percent of the total encapsulated [35S]parathion originally added to the elution column (5 mg). The flow rate was 0.27 mL/min. The experiment was repeated with different solvents with similar results. (D) Leaching of [35S]parathion expressed as a percent of the total encapsulated parathion originally added to the elution column; starch–xanthate capsules eluted with glass distilled deionized water (solid lines) or 1 N HCl (dashed lines) at 24.8 and 55 °C. Each line represents the summation of 44 separate determinations at intervals of 10, 70, or 280 min. The flow rate was 0.27 mL/min.

All treatments were initially wetted with 20 µL of artificial perspiration. After 24 h each skin disk was rinsed 5 times in water, fixed in 10% buffered formalin, dehydrated, embedded in paraffin, and sectioned. Sections of each treatment were stained with hematoxylin and eosin and by the periodic acid–Schiff (PAS) technique (Luna, 1968).

RESULTS

Both formulations stored well. In tightly capped vials there was no significant loss of [35S]parathion from either formulation over a 50-day period (Table I). Thin-layer radiochromatography revealed no change in the radiochemical purity of either formulation compared to the purity of the original stock [35S]parathion.

[35S]Parathion solubilized and volatilized less readily from SX. The leaching rate of [35S]parathion from SX was about half of that of AF when artificial perspiration was used as the eluting solvent (Figure 2A). The volatilization rate was significantly less for SX and wetting with artificial perspiration significantly increased the volatilization from both formulations (Figure 2B).

The leaching rate was inversely related to time and directly related to flow rate, H⁺ concentration, and temperature. In distilled deionized water (24.8 °C) the leaching rate decreased from 0.01.7%/min initially to 0.002%/min after 92.4 h (Figure 2C). Increasing the flow

Table I. Effect of Storage Time on Parathion Concentration in Starch-Xanthate Capsules (SX) and Adsorptive Formulation (AF)

time, days	concentration[a]	
	SX	AF
0	136 (4.35)	105 (11.35)
8	136 (2.65)	97 (1.70)
31	131 (2.84)	91 (9.80)
50	127 (4.89)	98 (3.12)

[a] Concentration = micrograms of parathion per milligram of capsules or adsorptive formulation. Values given as the mean with standard deviations in parentheses; n = 3. Both formulations were sized to < 20 but > 30 mesh and stored in tightly capped (with Teflon liners) glass vials.

rate resulted in an increase in the rate at which parathion leached from the capsules (Table II). After 92.4 h at 55 °C the total [35S]parathion solubilized was twice that released at 24.8 °C with distilled deionized water and use of 1 N HCl at 24.8 °C also doubled the total release relative to water at 24.8 °C (Figure 2d). In the presence of 1 N HCl at 55 °C the starch capsule hydrolyzed and abruptly released the [35S]parathion (Figure 2D).

Cutaneous penetration and accumulation of [35S]parathion were significantly less with SX in all three permeability experiments. While encapsulation reduced pene-

Table II. Effect of Flow Rate on Leaching of Parathion from Starch–Xanthate Capsules (SX) and Adsorptive Formulation (AF)

flow rate,[a] mL/min	mean % leached/min × 10^{-2} [b]	
	SX	AF
0.27	0.98 (0.07)	1.65 (0.15)
0.54	1.17 (0.04)[c]	2.17 (0.06)[c]
0.81	1.26 (0.04)[c]	2.33 (0.09)[c]
0.27	0.93 (0.04)	1.37 (0.16)

[a] Flow rates were initially 0.27 mL/min and then increased to 0.54 and 0.81 mL/min for 30 min at each flow rate and then decreased back to 0.27 mL/min. [b] Values given as the mean percent with standard deviations in parentheses; $n = 3$. [c] Significantly different from the initial elution rate at 0.27 mL/min; $P < 0.05$ (Student's t test).

Table III. Accumulation of Parathion in Skin after Contact with either the Starch–Xanthate Capsules (SX) or Adsorptive Formulation (AF)[a]

time, h	% initial dose	
	SX	AF
32	0.80 (0.70)	3.73 (1.33)
25	0.33 (0.20)	3.20 (0.90)
24	0.17 (0.024)	1.84 (0.52)

[a] After removal from diffusion vessel at the termination of the experiment, the skin was washed 5 times in distilled water. Values given are the mean ± standard deviation; $n = 3$. Results are from three separate experiments.

tration 2–4-fold (Figure 3), accumulation in the skin was reduced 4–10-fold (Table III). Thin-layer radiochromatography of benzene extracts of the receptor fluid and skin revealed that, with one exception, cutaneous accumulation and penetration did not result in production of detectable polar or nonpolar degradation products.

When wetted, skin treated with the SX became discolored and stiff relative to control skin and skin treated with AF. If artificial perspiration was allowed to evaporate to dryness, many of the starch–xanthate capsules became tightly adhered to the strateum corneum. This was not the case with the clay granules. Skin stained by the PAS technique (specific for polysaccharides) revealed that the strateum corneum of the skin treated with SX was thinly coated with PAS positive substances (Figure 4).

DISCUSSION

If it is assumed that the solubility, volatility, and permeability data for parathion can be generalized to other nonpolar pesticides of similar physical and chemical properties, then starch–xanthate-encapsulated pesticides are potentially much safer than clay-adsorbed formulations because of reduced toxicological risk from exposure via inhalation or cutneous penetration. Encapsulation reduces the inhalation risk by reducing the volatilization of the pesticide. Finely dispersed particles which might be generated during preparation, mixing, or application also pose less risk since reduced solubility and permeability will lessen the accumulated dose via the moist buccal and nasal epithelia, especially if these surfaces are washed soon after exposure. Additionally, relative to attapulgus clay granules, starch capsules are very hard and not easily fragmented or crushed. Therefore, handling of encapsulated pesticides will result in less fine dusts and thus reduce the probability of accumulation via the respiratory epithelia.

Since the main route of pesticide uptake is percutaneous (Dedek, 1980), the reduced solubility of pesticides in perspiration and other polar solvents and reduced cutaneous accumulation and penetration make starch–
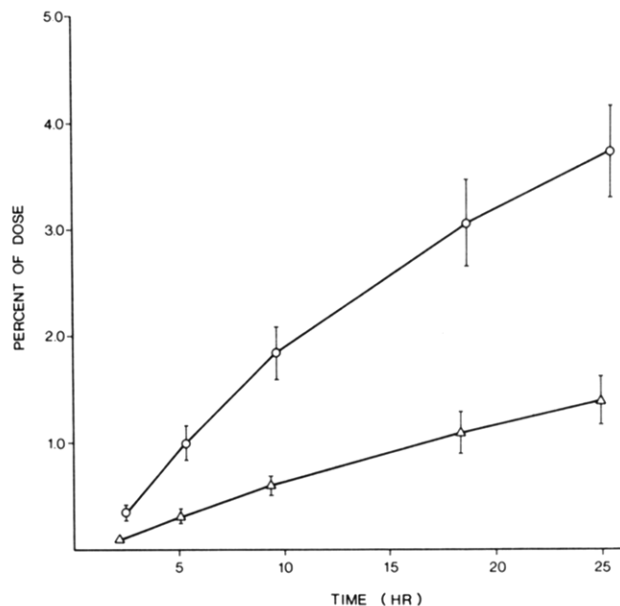
Figure 3. Accumulation of parathion in receptor reservoirs of diffusion cells: starch–xanthate capsules (triangles) and adsorptive formulation (circles). Results are expressed as the percent (mean ± standard deviation; $n = 3$) of the initial dose applied to the skin. This experiment was repeated a total of 3 times with similar results.
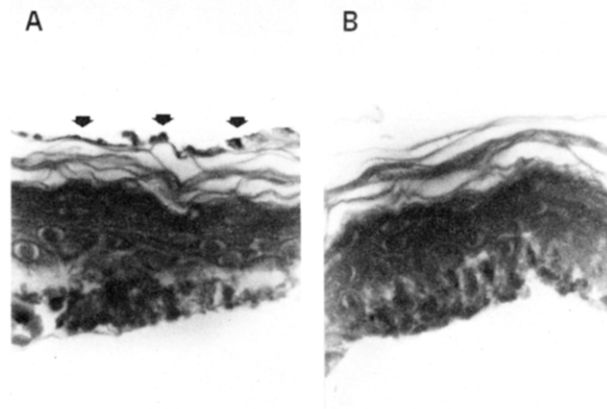
Figure 4. Cross section (87.5×) of skin disk from a 6-day-old suckling pig after 30-h exposure to (A) SX or (B) AF. Sections were stained by the periodic acid–Schiff technique. Arrows point out areas which stained positive for polysaccharides.

xanthate-encapsulated pesticides a much safer formulation even if protective clothing is worn. Often protective clothing can give false assurances of safety (Tordoir, 1980). When protective clothing made of organic polymer layers is worn, the penetration of nonpolar pesticides solubilized into polar solvents can be great (Dedek, 1980).

One characteristic of the starch–xanthate capsule which may reduce its safety, relative to the clay formulation, is its apparent tendency to coat the skin with starch and possibly other substances leached from the capsules. The tight adherence of capsules to the skin after drying could make the capsule and pesticide difficult to remove from skin and clothing.

With regard to environmental fate, the persistance of a starch–xanthate-encapsulated nonpolar pesticide, in any given soil system, will depend on such factors as starch digesting microbial activity (Otey, 1981), the amount of water percolation, H^+ concentration in the water, soil temperature, and aeration. A question which has not been addressed by this study, but is of importance from the

perspective of food-chain transfer, is the acceptability of the starch–xanthate capsules as food by nontarget animals such as birds and small mammals.

ACKNOWLEDGMENT

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Effects of Selected Insecticides and Herbicides on Free Sugar Contents of Carrots

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Early carrots were grown in soils treated or not with the insecticide Birlane; the carrots were covered or not covered with a plastic film; summer carrots were grown in soils treated or not with one of the insecticides Nexion, Birlane, or Dyfonate and with one of the herbicides Afalon Spezial or Dosanex. Several harvests were made; the main free sugars of the carrots, i.e., fructose, α- and β-glucoses, sucrose, and their total, were analyzed in the root of the carrot. Soil treatment with each of the three insecticides generally increased (relatively to the untreated soil) the concentrations of each of the free sugars and of their total in the root, the effect being the largest with Birlane and Nexion. The herbicide Afalon S generally had no effect on the sugar concentrations. The herbicide Dosanex generally decreased the concentrations of each of the free sugars and of their total.

Sweeney and Marsh (1971) reported the effects of two herbicides, the urea Afalon and the carbamate CIPC, on the carotene content of carrots grown in soil treated by one of these pesticides; the active matter of Afalon is linuron [3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea], and that of CIPC is chlorpropham (isopropyl 3-chlorophenyl-carbamate). Rouchaud et al. (1982a,b) reported the effects, on the total carotene content of carrots, of the organophosphorus insecticides Nexion, Birlane, and Dyfonate and of the urea herbicides Afalon Spezial and Dosanex; the active matter of Nexion is bromophos [O-(4-bromo-2,5-dichlorophenyl) O,O-dimethyl phosphorothioate]; that of Birlane is chlorfenvinphos [2-chloro-1-(2,4-dichlorophenyl)vinyl diethyl phosphate]; that of Dyfonate if fonofos [($\pm$)-O-ethyl S-phenyl ethylphosphonodithioate]; that of Afalon Spezial is a mixture of linuron [3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea] and monolinuron [3-(4-chlorophenyl)-1-methoxy-1-methylurea]; that of Dosanex is metoxuron [3-(3-chloro-4-methoxyphenyl)-1,1-dimethylurea]; the total carotene content was increased by the three insecticides, did not vary significantly with

Afalon S, and decreased with Dosanex.

The free sugars in the carrots are important comounds for their flavor quality (Alabran and Mabrouk, 1973). To our knowledge, there is no paper describing the influence of the pesticide treatments on the free sugars concentrations in carrots. The present work reports the concentrations of each of the free sugars in the early and in the summer carrots which were grown in soil treated with one of the five pesticides and in which the total carotene content was previously analyzed (Rouchaud et al., 1982a,b).

Exploratory analysis of the free sugars according to the method of Alabran and Mabrouk (1973) realized completely indicated that fructose, α- and β-glucose, and sucrose represented about 90–95% of the total of free sugars in the carrots analyzed here; for that reason, these sugars alone were searched by the routine analysis of the samples.

EXPERIMENTAL SECTION

Culture and Treatment of the Carrots. Early and summer carrot cultures were made at the Research Station for Vegetables, St-Katelijne-Waver, Belgium.

For the early culture, when treated, the soil was sprayed on Feb 2, 1981, with an aqueous emulsion of Birlane WP at the normal rate of 160 g of Birlane WP/are or at the exaggerated rate of 1600 g of Birlane WP/are. The

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