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Distribution and Elimination of [^{14}C]Malathion in the Rat

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The distribution pattern of malathion after intravenous administration of [^{14}C]malathion to rats was studied by whole-body autoradiography. Highest levels of radioactivity were detected in the liver and the kidney, which reached peak values 1–3 min following administration. The amount of radioactivity decreased rapidly, and after 24 h, only low levels in the liver, the kidney, the intestines, and the Harderian gland were detected.

The organophosphorus compound malathion, *O,O*-dimethyl *S*-(1,2-dicarbethoxyethyl) phosphorodithioate, has, compared to other organophosphates, a low mammalian toxicity and is widely used as a broad-spectrum contact insecticide and acaricide, as well as an ectoparasitic agent on both animals and human beings. Some investigations on the pharmacokinetic properties of malathion are published (Bourke et al., 1968; Gupta and Paul, 1976; Muan et al., 1985), but little has been done to clarify the distribution pattern of the compound. In the present work, the distribution in rats after intravenous administration of malathion has been studied by whole-body autoradiography and liquid scintillation counting.

MATERIALS AND METHODS

Test Materials. [^{14}C]Malathion, with specific activity 112 μCi/mg, was obtained from Amersham International plc (Buckinghamshire, England). It was prepared by the condensation of diethyl [2,3-$^{14}C_2$]maleate with *O,O*-dimethyl dithiophosphoric acid, and the radiochemical purity was 98%, as determined by thin-layer chromatography on silica gel in the manufacturer's laboratory. A 250-μCi portion of [^{14}C]malathion was dissolved in 1 mL of 96% ethanol and a salt buffer to 5 mL, to a final concentration of 50 μCi/mL.

Test Animals and Administration. Eight male Wistar rats (Møllegaard, Denmark) weighing 170–220 g were used. The animals were kept in cages, three in each, and maintained in 12 h light and 12 h darkness at 20 °C and 55% relative humidity. They were fed ad libitum with a commercial pelleted diet containing 24% proteins, 2% fat, and minerals and vitamins in adequate amounts (Møllesentralen I/S, P. Larsen & Co., Oslo, Norway) and had free access to water during the experiment.

The rats were given 200 μL/100 g of body weight (bw) of the solution of [^{14}C]malathion in the tail vein, corresponding to 10 μCi/100 g bw or 0.09 mg of malathion/100 g bw.

Whole-Body Autoradiography. One rat was sacrificed with diethyl ether euthanasia at 1, 3, and 10 min and 1, 2, 6, 12, and 24 h after administration. The animals were mounted in a 1% (w/v) aqueous gel of carboxymethylcellulose and frozen in a bath of *n*-hexane cooled with solid carbon dioxide to about −75 °C.

Sagittal sections (30 μm) through the whole frozen animal containing representative samples of all tissues were taken at different levels of the body. Three successive sections at each level were collected on tape (No. 821, 3 M Co., St. Paul, MN) at −20 °C in a PMV cryomicrotome (PMV 450 MP, Stockholm, Sweden). The sections were

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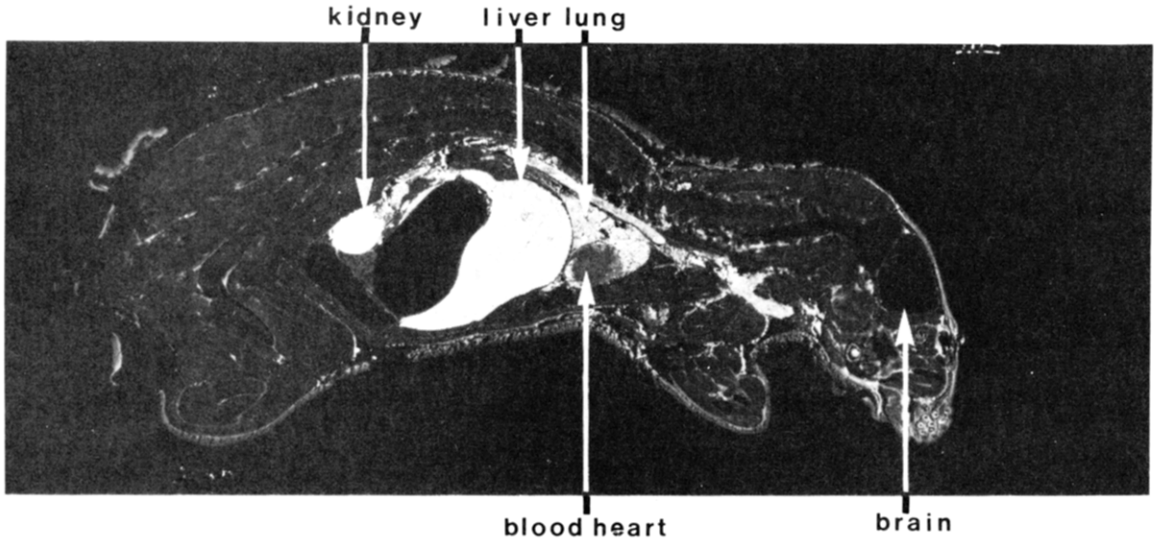

Figure 1. Whole-body autoradiogram of a male rat 1 min after an intravenous injection of [^{14}C]malathion. Light areas correspond to high concentrations of radioactivity.

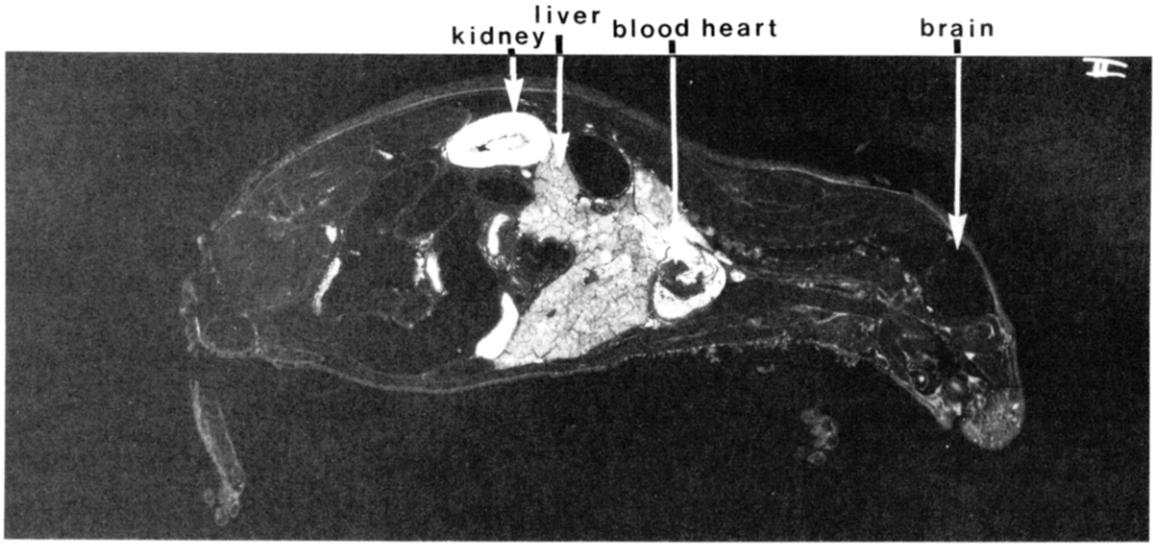

Figure 2. Whole-body autoradiogram of a male rat 10 min after an intravenous injection of [^{14}C]malathion.

freeze-dried at −20 °C for 24 h, and then placed in contact with X-ray films (Kodirex; Eastman Kodak, Rochester, NY). After exposure for 5 weeks, the films were developed and fixed. The procedure followed the autoradiographic method described by Ullberg (1954). In addition to visual inspection, the distribution of the radioactivity in the autoradiograms was evaluated by the densitometric method described by Cross et al. (1974). The sensory probe of the densitometer was held at a selected number of areas of each organ, and the relative amount of the isotope in different organs as compared to the liver was determined.

Liquid Scintillation Counting. Two parallels of 10-mg samples from randomly choosen areas of the liver, the brain, the kidney, the small intestine, and the large intestine were collected from the remaining of the frozen blocks. To each sample were added 200 μL of 96% ethanol and 1 mL of Soluene-350 tissue solubilizer (Packard, Zurich, Switzerland). Following incubation for 24 h at room temperature, 400 μL of 3% H_2O_2 (Perhydrol; Merck, Darmstadt, FRG) was added to decolorize the samples. After addition of 4 mL of scintillation fluid (Hionicfluor; Packard, Zurich, Switzerland) and equilibration at room temperature for 2 h, the samples were counted in a liquid scintillation spectrometer (Packard Tri-Carb 3310; Downers Grove, IL) for 10 min. Correction for quenching was made by use of autostandards. The results were expressed as disintegrations per minute (dpm) per 10 mg of tissue and calculated to give micrograms of malathion equivalents/gram of tissue.

RESULTS

Whole-Body Autoradiography. Autoradiograms prepared 1 min after administration of the [^{14}C]malathion (Figure 1) showed that the radioactivity already was distributed throughout most tissues. The highest activities were seen in the kidney, the liver, the lung, the heart, the skin, the musculature, and the blood. After 3 min, the distribution pattern was similar to that observed following 1 min. Ten minutes following the administration, the radioactivity in the liver had decreased and the highest activity was seen in the renal cortex, the medulla of the kidney, and the small intestine (Figure 2). As determined by densitometry (Table I), the activities in these tissues exceeded that of the liver by factors of 8, 2, and 2, respectively. Activities ranging from $^1/_{16}$ to 1 of that in the liver were observed in the brain, the blood, the heart, the lung, the musculature, the skin, and the large intestine.

The distribution patterns 1 and 2 h following the dosing were similar (Figure 3). The levels of activity in the liver and the blood were low compared to that of the renal

Table I. Radioactivity in Rat Tissues at Various Survival Times following Intravenous Administration of 10 μCi/100 g bw of [^{14}C]Malathion Assayed by Densitometry (Results Expressed as the Ratio of the Liver at the Corresponding Time Points)

time after admin	liver	renal medulla	renal cortex	brain	heart	small intestine	large intestine	blood	skin	lung	musculature
1 min	1	ND[a]	4	1/32	ND	ND	1/32	1/2	1/8	1/2	1/8
3 min	1	1/2	4	1/16	ND	1/4	ND	1/2	1/4	1	1/8
10 min	1	2	8	1/8	1/2	2	1/16	1	1/2	1	1/4
1 h	1	ND	4	1/8	1/2	1/4	1/8	1	1/2	1	1/4
2 h	1	ND	4	1/4	1/4	16	1/2	1/2	1/4	1/2	1/2
6 h	1	ND	2	1/4	1/4	1	16	1/2	1/4	1/2	1/4
12 h	1	2	4	1/4	ND	16	16	1/4	1/4	1/4	1/4
24 h	1	2	1	1/4	1/4	1/2	4	1/4	1/4	1/4	1/4

[a] ND = not determined.

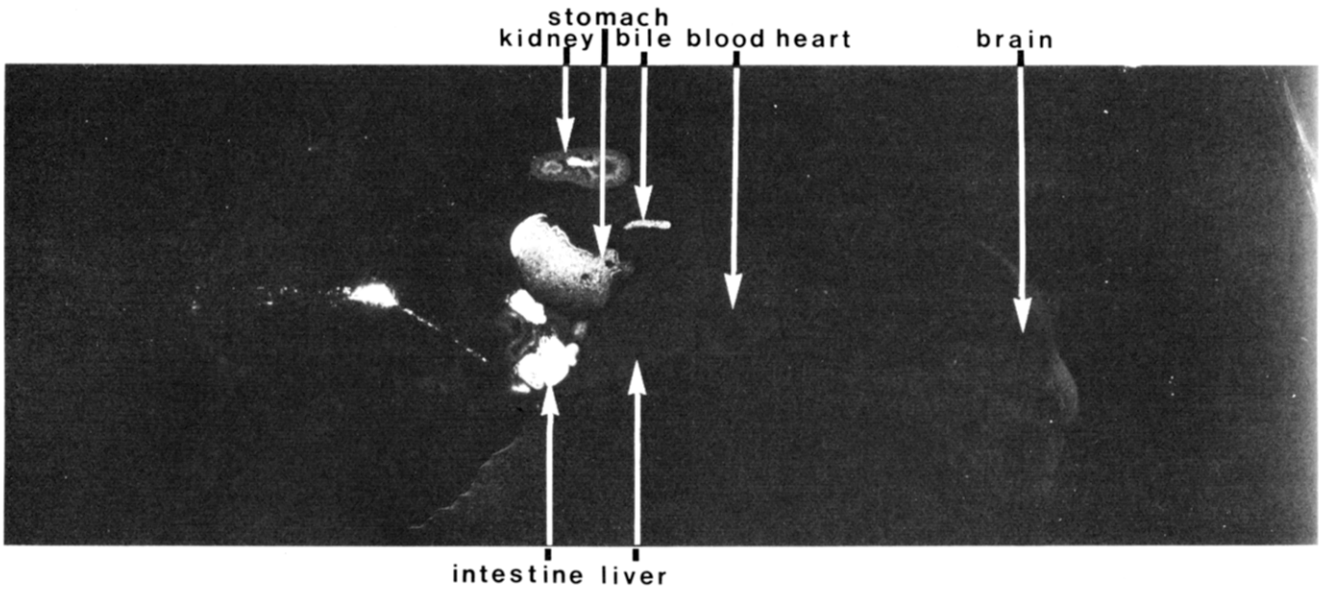

Figure 3. Whole-body autoradiogram of a male rat 2 h after an intravenous injection of [^{14}C]malathion.

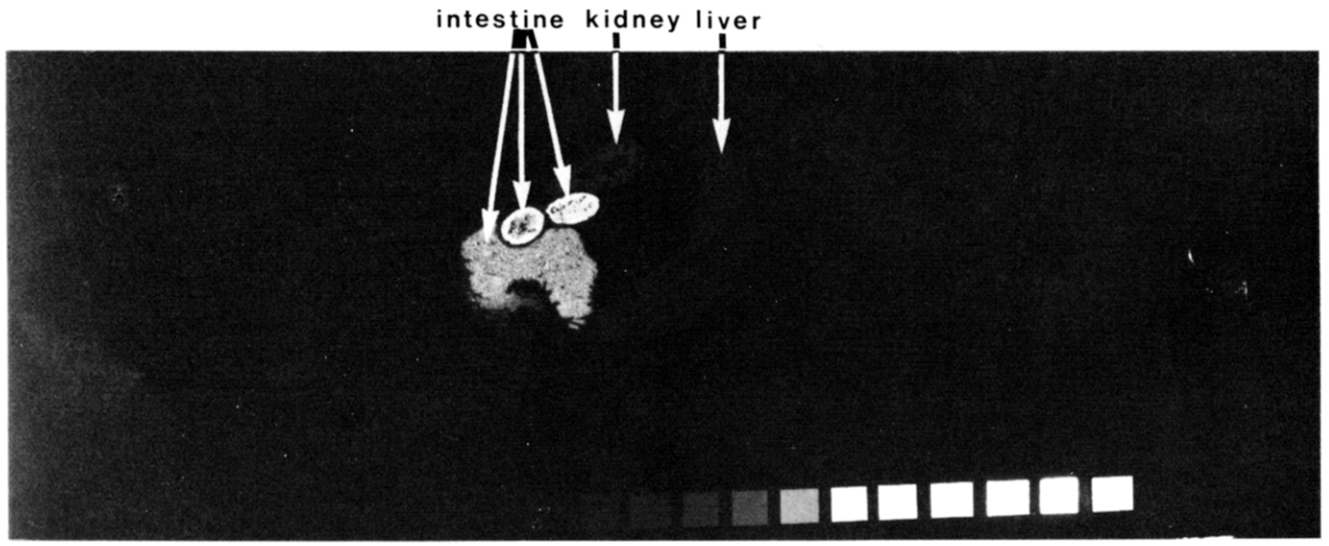

Figure 4. Whole-body autoradiogram of a male rat 12 h after an intravenous injection of [^{14}C]malathion.

medulla, the urine, the bile, the content of the ventricle, and the oesophagus. In the autoradiograms prepared after 6 h of survival time, the amount of radioactivity in most organs was about half that seen after 2 h, except for the large intestine where the activity had increased and had 16 times the activity of the liver.

At 12 and 24 h after dosing there were barely detectable levels of radioactivity in most organs, except the liver, the kidney, the intestines, and the Harderian gland (Figure 4). The activities in the liver and the kidney were low compared to the levels in the large intestine and the Harderian gland. At any time following the administration of radiolabeled malathion, almost no radioactivity could be detected in the central nervous system, the bone marrow, the testicles, or the endocrine glands.

Liquid Scintillation Counting. Results from the liquid scintillation counting are shown in Table II. The maximum levels of radioactivity in the kidney and the liver were found 1 and 3 min after administration, respectively. The peak values in the small and large intestines were found 1 and 6 h after dosing, respectively. These results were in agreement with the autoradiography.

Table II. Radioactivity in Rat Tissues at Different Survival Times following Intravenous Administration of 10 μCi/100 g bw of [^{14}C]Malathion Assayed by Liquid Scintillation Counting (Results Expressed as Micrograms of Malathion Equivalents/Gram of Tissue and Represent Values Obtained from Two Parallel Samples of 10 mg of the Tissues)

time after admin	liver	kidney	brain	small intestine	large intestine
1 min	3.38	12.35	0.05	0.00	0.11
3 min	4.05	2.51	0.25	0.73	0.01
10 min	1.21	8.98	0.11	1.31	0.01
1 h	1.19	0.98	0.13	4.19	0.07
2 h	0.30	0.73	0.00	0.12	0.29
6 h	0.00	0.34	0.00	0.00	0.38
12 h	0.00	0.00	0.00	0.00	0.29
24 h	0.00	0.07	0.00	0.00	0.02

DISCUSSION

Whole-body autoradiography gives a picture of the distribution of the total radioactivity administered and does not differentiate between the labeled compound and its metabolites. The results of the present study show that malathion is rapidly distributed to different organs in the rat, with the highest concentration found in the kidney. Peak levels of radioactivity in the liver and the kidney are reached within a few minutes following administration. After 1 h, the level in the kidney is reduced to about 5% of the maximum amount in that organ. After 24 h, the values of radioactivity are low in all organs. This indicates that the excretion of malathion is rapid. This is in agreement with the results published by Bourke et al. (1968). He found that after administration of 25 mg of [^{14}C]malathion to rats, activity appeared in the urine within 2 h and 91.7% was eliminated within 24 h, while an additional 7.75% remained in the gastrointestinal contents. Gupta and Paul (1976) have shown that over 90% of a single oral dose of malathion was excreted within 24 h in the hen. They found the highest concentration of malathion in the liver followed by the kidney and other organs. In a study on malathion used as an ectoparasitic agent on cattle, a rapid decline was demonstrated in the levels of malathion in blood and milk following an intravenous injection of the compound to lactating cows (Muan et al., 1985).

The presence of relatively high levels of radioactivity in the intestinal content may be due to bile excretion, mucosal secretion, or both. Eating of evacuation may be the explanation for the radioactivity detected in the esophagus and stomach. With the exception of the liver, the kidney, and the intestines, whole-body autoradiography revealed no particular accumulation organ for malathion.

Registry No. Malathion, 121-75-5.

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1-(4-Ethynylphenyl)-2,6,7-trioxabicyclo[2.2.2]octanes: A New Order of Potency for Insecticides Acting at the GABA-Gated Chloride Channel

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1-(4-Ethynylphenyl)-2,6,7-trioxabicyclo[2.2.2]octanes were prepared as candidate insecticides via palladium-catalyzed coupling of (trimethylsilyl)acetylene with the corresponding 1-(4-iodophenyl) compound or by dehydrobromination of the 1-[4-(1,2-dibromoethyl)phenyl] derivative. The 4-*tert*-butyl-1-(4-ethynylphenyl)trioxabicyclooctane has a topical LD_{50} for adult female houseflies (*Musca domestica* L.) of 0.06–0.09 μg/g alone or 0.01 μg/g on synergism with piperonyl butoxide at 25 or 35 °C. It is 20- to 40-fold more potent than previously reported 1,4-disubstituted trioxabicyclooctanes and is equal in potency to (1*R*,*cis*)-permethrin at 25 °C and (1*R*, *cis*,α*S*)-cypermethrin at 35 °C. This *tert*-butyl compound and its *n*-propyl and cyclohexyl analogues alone or with synergist are also much more potent than dieldrin, DDT, (1*R*,*trans*,α*S*)-allethrin, parathion, and propoxur. It therefore appears that suitable trioxabicyclooctanes acting at the GABA-gated chloride channel approach or reach the potency of the most effective established insecticides acting on sodium channels and the cholinergic system.

The 1,4-disubstituted 2,6,7-trioxabicyclo[2.2.2]octanes are a new class of insecticides that probably act as $GABA_A$ receptor antagonists and thereby inhibit GABAergic synaptic transmission (Palmer and Casida, 1985, 1987; Casida and Palmer, 1988). The compounds reported to date are very effective against houseflies and American cockroaches but only in the presence of piperonyl butoxide (PB), indicating that their toxicity is limited by oxidative detoxification.

The present study attempts to improve the insecticidal activity of trioxabicyclooctanes and minimize the need for a synergist by appropriate modifications of substituents in the 1- and 4-positions. Houseflies are used for the primary bioassays because of the available structure–activity data with this species. A more general goal is to

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