Evaluation of Diethyl Malonate as a Simulant for 1,2,2-Trimethylpropyl Methylphosphonofluoridate (Soman) in Shower Decontamination of the Skin

WILLIAM G. REIFENRATH *x, MILLARD M. MERSHON ‡, FLOYD B. BRINKLEY ‡, GEORGE A. MIURA ‡, CLARENCE A. BROOMFIELD ‡, and H. BRUCE CRANFORD §¶

Received August 16, 1983, from the **Division of Cutaneous Hazards, Letterman Army Institute of Research, The Presidio, San Francisco, CA 94129*, ‡*U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD 21010*, and the §*U.S. Army Medical Bioengineering R&D Laboratory, Fort Detrick, Frederick, MD 21701.* Accepted for publication October 20, 1983. ¶ Present address: U.S. Department of Energy, Office of Industrial Programs, Washington, DC 20585.

Abstract □ A shower decontamination bench model has been used to assess quantitatively the importance of several variables (water pressure and temperature, surfactant concentration in the decontamination fluid, nozzle type, and shower time) on decontamination of nontoxic chemical warfare-agent simulants diethyl malonate and thickened diethyl malonate from pig skin *in vitro*. Diethyl malonate was validated as a simulant for 1,2,2-trimethylpropyl methylphosphonofluoridate (soman) by comparison of the skin penetration and decontamination of radiolabeled diethyl malonate to the radiolabeled phosphonofluoridate in shower decontamination trials of pig skin *in vitro*. Percutaneous penetration of diethyl malonate was significantly greater than that of the phosphonofluoridate during the 15-min period after application. However, both were <0.1% of the applied dose. Showering or thickener had no significant effect on the percutaneous penetration of diethyl malonate or the phosphonofluoridate. Most of the phosphonofluoridate removed by showering or scrubbing the skin was inactivated. The quantity of intact 1,2,2-trimethylpropyl methylphosphonofluoridate that penetrated through the skin was below the detection limit of the enzymatic analysis. There was no statistically significant difference between the phosphonofluoridate and diethyl malonate in efficacy of shower decontamination. The presence of thickener did not have a significant effect on decontamination efficacy.

Keyphrases □ Shower decontamination—percutaneous absorption, pig skin □ Diethyl malonate—percutaneous absorption, pig skin □ 1,2,2-Trimethylpropyl methylphosphonofluoridate—percutaneous absorption, pig skin

In the event of skin exposure to toxic chemicals, involved armed forces and civilian groups will need to decontaminate nonambulatory chemically contaminated casualties before they receive medical treatment for wounds. Besides decontamination of the patient, another purpose of decontamination is to protect medical personnel and prevent their exposure to detrimental levels of toxic chemicals. It is generally not possible for medical personnel in protective clothing to provide necessary medical treatment to patients. Consequently, medical personnel must operate in a shirt-sleeve environment.

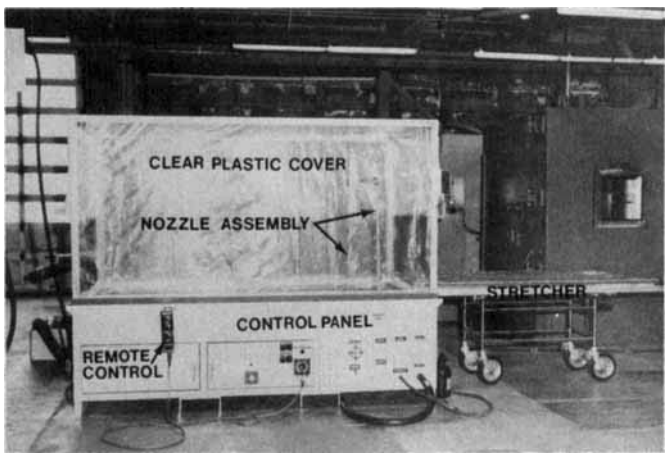

Figure 1—*United States Air Force litter patient decontamination shower (breadboard model).*

Equipment designers do not have sufficient information on nonambulatory casualty decontamination to construct a prototype device for deployment and installation in fixed facilities. To obtain the necessary information, a breadboard model (an experimental item of hardware fabricated during the conceptual phase to reduce technological uncertainty, prove feasibility, and provide realistic cost estimates) of a decontamination device has been fabricated by the U.S. Army Medical Bioengineering Research and Development Laboratory (Fig. 1). The decontamination device is conceived to work as follows. The chemically contaminated patient is placed on a stretcher where all clothes, dressings, *etc.* are removed and the patient is rolled into the decontamination device. (During the entire process, the gas mask will remain on the patient.) The device is surrounded by a clear plastic cover which allows observation of the patient, permits easy access to the patient, and prevents escape of contaminated water. The device, activated and controlled by an operator, can apply soap and water (or other chemicals) to the entire body, except the area under the mask. With the patient stationary, the nozzle assembly cycles back and forth, applying water, soap, and rinse water for a predetermined time. When decontamination is considered complete, the clean side of the machine is opened and the stretcher with patient is removed. Additional background information on the Litter Patient Decontamination Shower is available (1).

In a previous study (1), an assessment was made of the effect of variables functioning in the breadboard device (water pressure, surfactant concentration, water temperature, nozzle

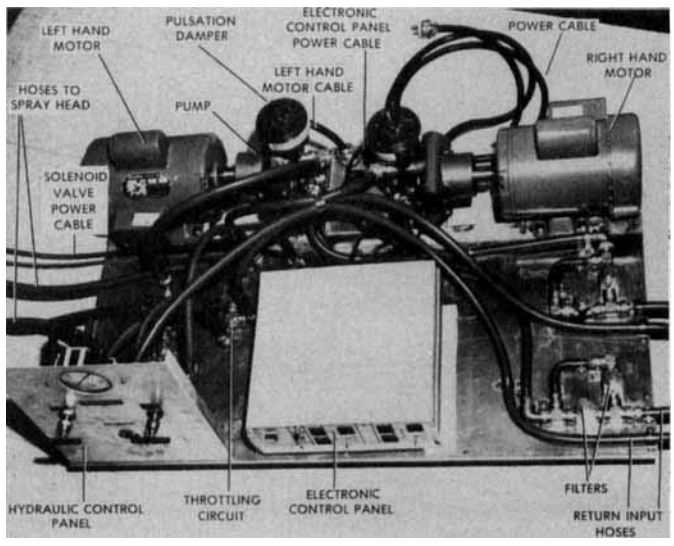

Figure 2—*Litter patient decontamination unit, bench model; top view showing dual water circuits.*

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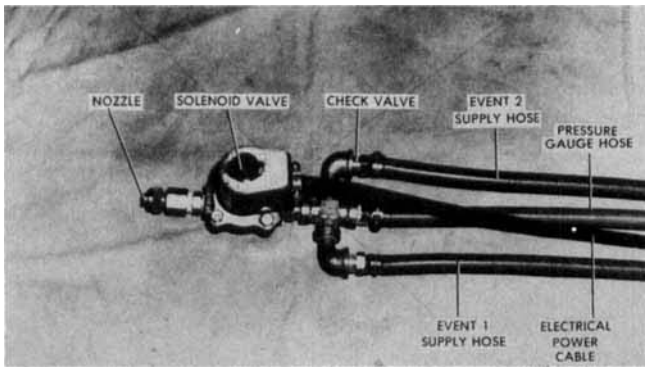

Figure 3—*Litter patient decontamination unit, bench model; shower head and connections are shown.*

type, and shower time) on removal of nontoxic chemical warfare agent simulants from the surface of excised pig skin. Diethyl malonate was used as a simulant for the nerve agent 1,2,2-trimethylpropyl methylphosphonofluoridate (soman), and diethyl malonate thickened with acrylate polymer was used as a simulant for the thickened phosphonofluoridate. Diethyl malonate was chosen because its physical properties (vapor pressure, water solubility, and surface tension) are similar to those of the phosphonofluoridate (1). A decontamination bench model (Figs. 2 and 3) was used to simulate the function of the breadboard decontamination device. The bench model is a single stationary nozzle model of the breadboard device. In the previous report (1), it was found that the force per unit area exerted by the shower water on the skin surface was the major variable responsible for differences in decontamination efficiency. Compared with trials where no showering was done, showering always increased the mean percutaneous penetration of simulant. However, the significance of these results was in question since the effect was small and varied in magnitude and the ability of diethyl malonate to simulate the percutaneous penetration of the phosphonofluoridate was unknown. Therefore, the present study was conducted to compare the percutaneous penetration of radiolabeled diethyl malonate and radiolabeled 1,2,2-trimethylpropyl methylphosphonofluoridate (both unaltered and in the thickened state) in shower decontamination trials of pig skin *in vitro*.

EXPERIMENTAL SECTION

Radiolabeled and Thickened Radiolabeled Diethyl Malonate—Diethyl [2-^{14}C]malonate[1] had a radiochemical purity of 98%; diethyl malonate[2] had a purity of 99%. Radiolabeled diethyl malonate was diluted with unlabeled diethyl malonate to give a specific activity of 5 μCi/mg. Applying 0.27 μL of this dilution to a skin area of 2.85 cm^2 gave a chemical dose of 0.1 mg/cm^2 and a radioactive dose of 1.35 μCi. A sample of thickened radiolabeled diethyl malonate was prepared by adding 20 mg of radiolabeled diethyl malonate to 100 μL of a mixture of acrylate polymer[3] in unlabeled diethyl malonate with a viscosity of 1000 centistokes at 25°C to give a final viscosity of ~200 centistokes at 25°C. Applying 0.28 μL of this mixture to a skin target area of 2.85 cm^2 gave a chemical dose of 0.1 mg/cm^2 and a radioactive dose of 1.76 μCi.

Preparation of Radiolabeled and Thickened Radiolabeled 1,2,2-Trimethylpropyl Methylphospbonofluoridate—Into a 12-mL capped centrifuge tube equipped with a triangular stirring bar was placed 30 μL of triethylamine, 1 mmol (~125 μL) of [1-methyl-^{14}C]3,3-dimethyl-2- butanol[4], and an excess

[1] Batch no. 53, specific activity 4.9 mCi/mmol; Amersham Radiochemicals, Arlington Heights, Ill.
[2] Lot no. 47C-0374; Sigma Chemical Co., St. Louis, Mo.
[3] Polymer K125EA, a polymer made from the following monomers: methyl methacrylate, ethyl acrylate, and butyl acrylate; Rohm and Haas Co., Philadelphia, Pa.
[4] Lot no. 710674, specific activity 5 mCi/mmol; ICN, Chemical and Radioisotope Division, Irvine, Calif.

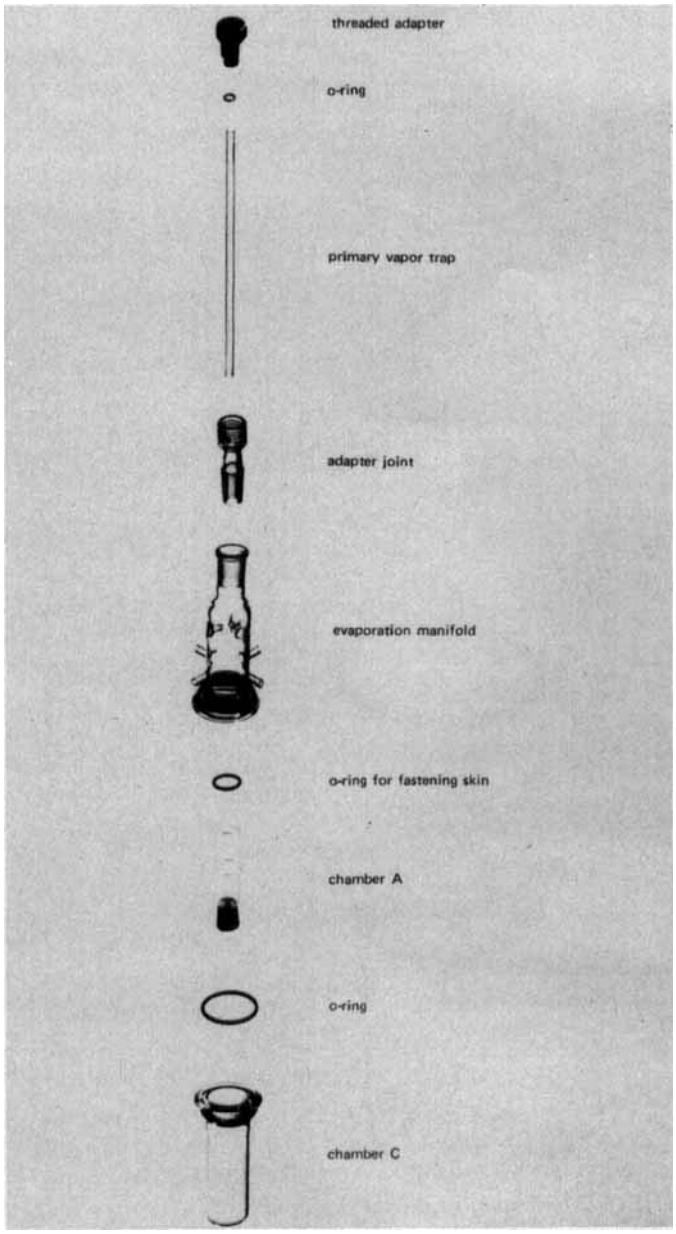

Figure 4—*Apparatus for collection of diethyl malonate and phosphonofluoridate evaporation.*

(~200 μL, 2 mmol) of methylphosphonic difluoride, of which 100 μL was used to rinse the residual pinacolyl alcohol from the vial before it was added to the centrifuge tube. The resultant mixture was allowed to stand at room temperature overnight. One milliliter each of distilled water and $CDCl_3$ were added, the mixture was shaken vigorously, and then centrifuged in a clinical centrifuge[5] at one-half speed for 2 min. The aqueous layer was carefully removed with a pipet, and another 1 mL of distilled water was added. The mixture was shaken and centrifuged as before, the top layer was again carefully removed, and the organic layer was transferred to a flask with molecular sieves. After drying overnight the solution was transferred to an NMR tube for analysis. By ^{1}H- and ^{31}P-NMR, the sample contained 75–82% of the phosphonofluoridate; the remainder was 3,3-dimethyl-2-butanol. The sample was distilled to yield a homogeneous sample by TLC[6] (5% methanol–chloroform) with single spot at R_f 0.82; all of the radioactivity was associated with this single spot.

The radiolabeled phosphonofluoridate was diluted with unlabeled compound so that when 0.27 μL was applied to a 2.85 cm^2 skin area, a chemical dose of 0.1 mg/cm^2 and a radioactive dose of 0.9 μCi were given. A sample of the thickened radiolabeled phosphonofluoridate was prepared by adding 20 mg of radiolabeled compound to 100 μL of a mixture of acrylate polymer

[5] International Equipment Company, Needham Heights, Mass.
[6] Silica gel sheet no. 6061; Eastman Organic Chemicals, Rochester, N.Y.

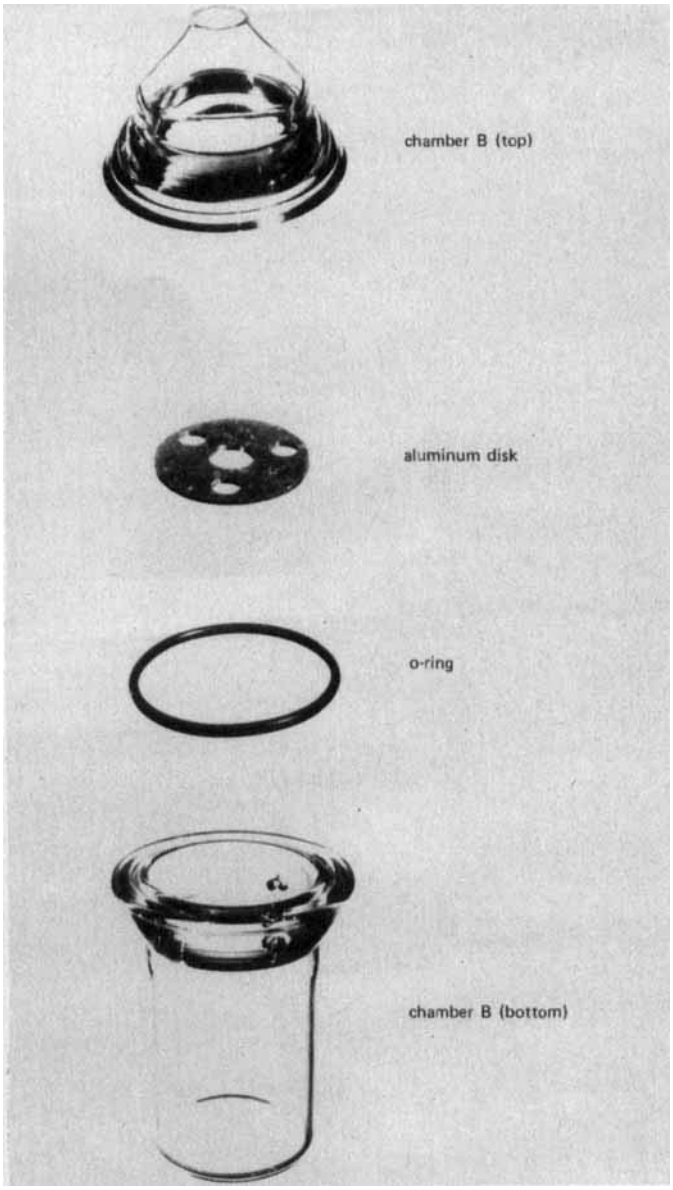

Figure 5—*Apparatus for collection of shower water.*

and unlabeled phosphonofluoridate (1000 centistoke at 25°C) to give a final viscosity of ~200 centistokes at 25°C. Applying 0.28 μL of this mixture to a 2.85 cm^2 skin area gave a chemical dose of 0.1 mg/cm^2 and a radioactive dose of 1.6 μCi.

Acetylcholinesterase Inhibition Assay[7]—Inhibition of acetylcholinesterase activity served as the basis for the enzymatic assay of the ^{14}C-labeled phosphonofluoridate. Enzyme activity was measured by the radiometric method of Siakotos *et al.* (2).

Acetylcholinesterase Stock Solution For Enzymatic Assay of the Phosphonofluoridate—Ten milligrams of eel acetylcholinesterase and 500 mg of bovine plasma albumin were dissolved in 500 mL of 10 mM potassium phosphate buffer, pH 7.0. Aliquots were stored at −20°C. Prior to use, an aliquot of enzyme solution was thawed, and $MgCl_2$ and NaCl were added to a final concentration of 10 mM and 100 mM, respectively.

Preparation of Dilutions and Determination of the Standard Curve—1,2,2-Trimethylpropyl methylphosphonofluoridate (20 mg) was dissolved in 100 mL of normal saline. The initial dilution was made by adding 100 μL of a 1.1 mM stock solution of the phosphonofluoridate to 0.90 g of ice-cold glass-distilled water in ~1.9-mL screw-top vials. Stock solutions (1.1×10^{-8} and 2.2×10^{-8} M) were prepared by serial dilution and kept on ice. Fresh

[7] [^{14}C]Acetyl-β-methylcholine and aqueous scintillation counting fluid (Aquasol) were purchased from New England Nuclear Corp., Boston, Mass. Eel AchE, type VI, was a product of Sigma Chemical Co., St. Louis, Mo. Amberlite CG-120 resin (sodium form, 200–400 mesh) was obtained from Mallinckrodt Co., St. Louis, Mo. Bovine plasma albumin was obtained from Armour Laboratory, Chicago, Ill.

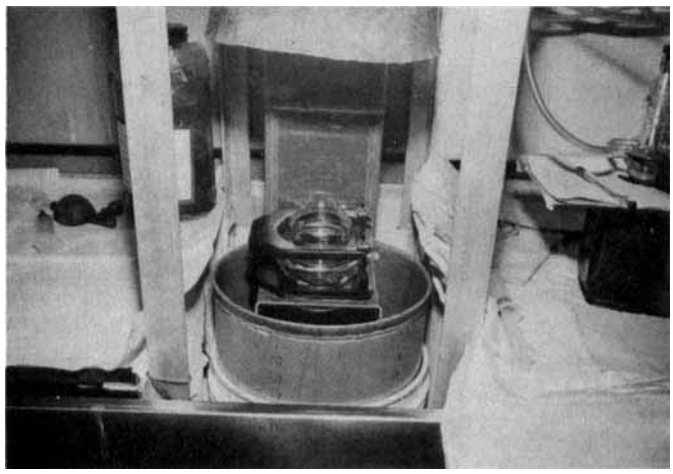

Figure 6—*Shower target assembly mounted in a hood.*

dilutions were prepared daily, and the standard curve was determined immediately after serial dilution. Assays were performed in 12-mL graduated centrifuge tubes. Four replicates were prepared for each concentration of the phosphonofluoridate. The reaction mixture contained 20 μL of 0.5 M potassium phosphate buffer (pH 7.4), 100 μL of eel acetylcholinesterase stock solution, 10 μL of 300 mM $MgCl_2$, the phosphonofluoridate solution, and enough glass-distilled water to make 210 μL. The mixture was incubated for 15 min at 37°C. To this mixture was added 100 μL of 3 mM [^{14}C]acetyl-β-methylcholine (0.1 μCi/100 μL), and the final mixture was incubated for 3 min at 37°C. The reaction was stopped by the addition of 5 mL of 20% (w/v) resin suspension in dioxane. The volume of each stopped-reaction mixture was adjusted to 10 mL with dioxane. After centrifugation, a 5-mL aliquot of dioxane supernatant (containing [^{14}C]acetate) was added to 10 mL of aqueous counting solution. Radioactivity was determined using a scintillation counter[8] which corrected for quenching. Radioactivity from controls, which were incubated with 100 μL of water in place of acetylcholinesterase, was subtracted from all assays to correct for nonenzymatic hydrolysis of the substrate. Inhibition of enzyme activity was expressed as the percentage of control which was not treated with the phosphonofluoridate.

Dilution of Radiolabeled Phosphonofluoridate Samples for Quantitative Enzyme Determination—Samples of solutions of the labeled phosphonofluoridate were kept on ice and diluted serially when necessary with ice-cold glass-distilled water so that appropriate aliquots inhibited the acetylcholinesterase at levels on the linear portion of the standard curve. Assays of the samples were carried out immediately. The procedures described above for the standard curve were followed for the label phosphonofluoridate samples. Stability of the radiolabeled compound was assessed before, during, and after the completion of the study by the enzymatic procedure. Initial and midpoint purities were essentially 100%. Purity was 70% when determined after the completion of the experiments.

Skin Samples—Skin was harvested from each of three female weanling Yorkshire pigs (~25 kg)[9]. After the pigs were sacrificed, hair was clipped from the animals with electric clippers[10]. An incision (~30 cm) was made down the midline of the back. At the 0-, 15-, and 30-cm points along the midline incision, perpendicular incisions (~15 cm) were made on the left side of the animal. A final incision was made parallel to the initial midline incision so that two square sections (15 cm × 15 cm) were formed. A notch was cut in the anterior edge of each section to preserve orientation. Whole-skin sections were removed from the animal and subcutaneous fat was removed by scraping. A small piece of skin from each animal was submitted for histopathological examination, which verified that the skin was normal tissue. The skin was cut into 3.2-cm diameter circles by using a punch. Each skin sample was coded by location. Skin was stored at −60°C for ~1 month prior to use.

Apparatus—Special glassware (Figs. 4 and 5) used in the study was custom-fabricated[11]. The bench-model decontamination unit was fabricated at the U.S. Army Medical Bioengineering R & D Laboratory, Fort Detrick, Frederick, Md. Details of the bench pump design can be found elsewhere (1). An assembly was constructed inside a hood (minimum linear air velocity of 45.8 m/min) to position the shower head of the bench model 30.5 cm above the skin surface when the skin was mounted on chamber A and inside chamber

[8] Model 2660; Packard Instrument Co., Des Plaines, Ill.
[9] Boswell Laboratory Animals, Corcoran, Calif.
[10] Model no. A2 small clipper; Oster Corp., Milwaukee, Wis.
[11] Laboratory Glass Apparatus, Berkeley, Calif.

Table I—Disposition of Radioactivity Following Application of Radiolabeled Diethyl Malonate and 1,2,2-Trimethylpropyl Methylphosphonofluoridate and Thickened Radiolabeled Compounds to Pig Skin

Chemical	Condition[b]	Applied Radioactive Dose, %[a]				
		Evaporation[c]	Decontamination[d]	Scrub[e]	In Skin[f]	Penetration[g]
Diethyl malonate	No shower	63.1 ± 3.6	—	13.8 ± 2.0	2.4 ± 0.7	0.09 ± 0.03
Phosphonofluoridate	No shower	61.0 ± 7.1	—	5.3 ± 1.1	1.1 ± 0.3	0.03 ± 0.02
Diethyl malonate	Shower	54.9 ± 13.7	13.6 ± 3.2	2.1 ± 0.8	2.3 ± 0.5	0.10 ± 0.05
Phosphonofluoridate	Shower	58.0 ± 12.8	4.6 ± 2.2	1.1 ± 0.5	0.6 ± 0.2	0.03 ± 0.02
Thickened diethyl malonate	No shower	53.9 ± 7.8	—	10.3 ± 1.1	2.2 ± 1.4	0.08 ± 0.05
Thickened phosphonofluoridate	No shower	60.4 ± 8.8	—	5.7 ± 2.6	0.8 ± 0.2	0.03 ± 0.02
Thickened diethyl malonate	Shower	52.1 ± 10.4	9.6 ± 1.7	1.9 ± 1.0	1.9 ± 1.0	0.06 ± 0.04
Thickened phosphonofluoridate	Shower	60.4 ± 4.9	5.0 ± 2.2	1.6 ± 0.5	0.9 ± 0.5	0.01 ± 0.01

[a] Data expressed as mean ± *SD* for six replicate determinations. [b] Shower conditions consist of two 1-s bursts of water at a theoretical force per unit area of 0.054 kg/6.45 cm^2 on the skin surface. [c] Radioactivity recovered from the primary vapor trap. [d] Radioactivity removed from the skin by showering. [e] Radioactivity removed from the skin by scrubbing and rinsing the skin surface with 1% aqueous surfactant solution. [f] Radioactivity recovered by oxidation of the skin sample. [g] Radioactivity recovered from chamber A.

B (Fig. 6). The entire shower target assembly was surrounded by a movable shield to control water spray.

Shower Decontamination Trials—The skin disposition of diethyl malonate was compared with that of the phosphonofluoridate, and the skin disposition of thickened diethyl malonate was compared with that of the thickened phosphonofluoridate in shower decontamination trials. These experiments were compared with experiments where procedures remained the same except that no showering was done. Eight combinations of variables, which represented all possibilities, were compared on 6 separate days (48 total trials). The order in which the eight combinations were tested on a given day was randomized. Skin samples from the two sites on each of the three pigs were allocated to the experimental trials in a random fashion, with the restriction that an equal number of samples came from each of the three pigs.

Skin samples were removed from the −60°C freezer to a −8°C freezer the night before trials were made. The skin was placed in a glass vial in a 37°C water bath and allowed to thaw (15–20 min); the weight of the skin and location code were recorded. The thawed skin was attached to chamber A (Fig. 4) with a rubber O-ring. Chamber A was filled with Ringer's lactate, prewarmed to 37°C, and stoppered. Chamber A was placed inside chamber C, both within a water bath at 37°C. After the skin surface temperature reached 25–27°C, the skin surface was dosed with the appropriate chemical by streaking it across the skin surface (2.85 cm^2) with the blunt tip of a 1-μL syringe[12]. Immediately following application, the evaporation manifold was clamped to chamber C with chamber C remaining in the 37°C water bath. A peristaltic pump[13] was connected to the primary vapor trap (a tube containing 200 mg of absorbent powder[14]). The pump pulled air at 60 mL/min, causing air to flow into the inlet tubes of the evaporation manifold, over the skin surface, and into the primary vapor trap. Any radiolabeled chemical that evaporated from the skin surface was absorbed by the primary trap. A secondary safety bubbler trap was connected between the pump and the primary vapor trap.

After 14 min, chamber A was removed and mounted into chamber B (Fig. 5). Fifteen minutes after application of chemical, the skin was showered. The bench model was designed to shower the skin with a decontamination fluid followed by a water rinse. However, in a previous comparison (1) involving removal of thickened and unthickened diethyl malonate from skin, no difference was found between cycles with a 1% aqueous surfactant solution and with plain water. Therefore, distilled water was used in both shower cycles of this study. A total shower time of 2 s was used, 1 s/cycle. The water temperature was 20°C, and the pH was 6.8. After showering, chamber A was removed from chamber B. The sides of chamber A were rinsed into a tared beaker along with the contents and several rinses of chamber B. While tilting chamber A downward, the skin surface was scrubbed with a cotton ball containing 1% aqueous surfactant[15] and rinsed with 1 mL of the 1% aqueous surfactant (pH 6.7). The skin rinse and cotton ball were collected in a counting vial and assayed for radioactivity. The contents and rinses of the interior of chamber A were collected in a tared beaker and assayed for radioactivity (percutaneous penetration). The skin was removed from chamber A and cut into seven approximately equal portions. These were later assayed for radioactivity by combusting the samples[16], trapping the liberated $^{14}CO_2$[17], and counting the radioactivity on a scintillation counter. The primary vapor trap was removed from the evaporation mainfold and the contents (chemical trapped on absorbant powder) were flushed with aqueous (scintillation) counting fluid rinses (~15 mL each) into a counting vial. The content of this counting vial was then divided into six additional counting vials for more accurate counting. For each day, the volume of substance applied to the skin was placed in each of three volumetric flasks to serve as a control; an aliquot was taken from each for radioactivity assay.

When samples of the radiolabeled and thickened radiolabeled phosphonofluoridate were used, aliquots of residues recovered by scrubbing the skin surface, and from chamber A and chamber B, were subjected to the enzymatic assay.

RESULTS AND DISCUSSION

The disposition of radioactivity applied to the skin under the various conditions of the study is given in Table I. Overall recovery of the radioactive dose in these trials was ~70%, with the major portion recovered after evaporation from skin. The balance of the radioactive dose was probably lost by evaporation during the skin application process, before the evaporation manifold was in place. The amounts of evaporated diethyl malonate and 1,2,2-trimethylpropyl methylphosphonofluoridate, as measured by the amount of radioactivity recovered from the primary vapor trap, were very similar. This result was not changed by the presence of thickener.

A higher percentage of the radioactive dose of diethyl malonate than the phosphonofluoridate was recovered from the skin surface (by scrubbing) and from residues in the skin (by oxidation) at the end of 15 min following application without showering. However, showering removed a correspondingly larger percent of the applied radioactive dose of diethyl malonate than phosphonofluoridate so that residues left on the skin surface were similar (1–2%). Again, the presence of thickener did not change this outcome.

The efficiency of shower decontamination (calculated by dividing the percentage in the "Decontamination" column in Table I by the sum of percentages in the last four columns and multiplying by 100) showed little difference between the four test substances as evidenced by the following values (mean ± *SD*): diethyl malonate, 75 ± 7; phosphonofluoridate, 69 ± 14; thickened diethyl malonate, 72 ± 21; thickened phosphonofluoridate, 65 ± 13.

In a previous study (1), it was found that the force per unit area exerted by the shower on the surface of pig skin was the major variable responsible for differences in decontamination efficiency of diethyl malonate and thickened diethyl malonate. Varying the temperature of water from 23°C to 37°C or adding surfactant to the water had little effect on the decontamination efficiency (1). These results, together with the findings that all four test substances were decontaminated equally by the shower, suggest that the mechanism of shower cleaning is mechanical rather than chemical. Apparently, the shower cleans the skin by removing the upper layers of the stratum corneum so that contaminants residing in these upper layers are removed. The velocity of the water droplets in the shower could also contribute to mechanical removal of contaminants not in direct contact with the skin.

During the 15-min period immediately following application the percutaneous penetration of diethyl malonate, as measured by radioactivity appearing in chamber A, was significantly greater than the percutaneous penetration of the phosphonofluoridate (Table I). However, both were <0.1% of the applied dose. An analysis of variance revealed that showering or thickener had no significant influence on the percutaneous penetration of diethyl malonate or the phosphonofluoridate.

Enzymatic analysis showed inactivation of most of the phosphonofluoridate represented by carbon-14 removed from skin by showering or scrubbing (Table II). Any phosphonofluoridate that had penetrated through the skin was below the detection limit of the enzymatic analysis. The purity of the stock samples of the radiolabeled phosphonofluoridate was checked initially, during the

[12] No. 7101; Hamilton Co., Reno, Nev.
[13] Masterflex, with Model 7014 head; Cole Parmer Instrument Co., Chicago, Ill.
[14] Tenax GC; Alltech Associates, Arlington Heights, Ill.
[15] Triton X-100; Rohm and Haas Co.
[16] Model 306 sample oxidizer; Packard Instruments.
[17] Carbosorb; Packard Instruments.

Table II—Enzymatic Analysis of Skin Decontamination Water and Skin Scrub Solution

Chemical	Condition	Active Agent, %[a] Decontamination Water[b]	Active Agent, %[a] Scrub Solution[c]
Phosphonofluoridate	No shower	—	13 ± 12
	Shower	15 ± 6	17 ± 6
Thickened phosphonofluoridate	No shower	—	8 ± 7
	Shower	16 ± 13	15 ± 15

[a] Percent of recovered radioactivity associated with active agent as determined by enzymatic assays. Numbers are mean ± *SD*. [b] Water collected in chamber B after shower decontamination of the skin. [c] One percent aqueous surfactant solution used to scrub and rinse the skin.

course of the study, and after the completion of the experiments. Purity was essentially 100% at the initial and midpoint, but fell to 70% after the completion of the experiments. However, no significant differences were found between results from initial and final samples.

The inactivation of 1,2,2-trimethylpropyl methylphosphonofluoridate in contact with skin is consistent with the observations of Fredricksson (3) who demonstrated the presence of organophosphate ester hydrolases in the skin of rats. Other investigators (4) who compared skin penetration of the phosphonofluoridate activity with radiolabel penetration reported that human skin decomposed at least 80% of the applied phosphonofluoridate as contrasted with 30% inactivation of its penetration of guinea pig skin.

Diethyl malonate was selected as a simulant for the phosphonofluoridate in skin-surface removal studies on the basis of similar physical properties. For both compounds, the majority of the applied dose evaporated. After 15 min, most of the radiolabel that remained (Table I) was recovered from the skin surface (by showering or scrubbing). The lack of agreement between radioactivity and enzyme inhibition assays of the shower and scrub water would suggest that some of the phosphonofluoridate hydrolyzed after it contacted the skin. In the case of diethyl malonate, hydrolysis on the skin is certainly possible. It is, therefore, possible that the shower removed a mixture of the original compounds as well as hydrolysis products, and it is no longer clear what is being compared. Presumably, the intact compound would have a different affinity for the skin than its hydrolysis products. The fact that similar skin decontamination efficiencies (as judged by the percent removal of radioactivity still on the skin after evaporation) were obtained by showering after application of the radiolabeled diethyl malonate and phosphonofluoridate may be fortuitous. However, if the shower removed the upper layers of skin, and thus removed contaminants in these layers indirectly, decontamination of surface residues would be independent of chemical structure, and similar decontamination efficiencies would be obtained as in this study.

Under the conditions of this study, skin penetration of 1,2,2-trimethylpropyl methylphosphonofluoridate and diethyl malonate was limited by the dose, short interval between application and decontamination, and the free evaporation of the chemicals during this interval. The skin penetration of the phosphonofluoridate is complicated by the possibilities of chemical degradation during its passage through the skin. Attempts to compare the percutaneous penetration of this phosphonofluoridate (and other highly toxic organophosphorous compounds) to analogues or simulants must await knowledge of the fate and distribution of these compounds in skin.

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Stereospecific Fluorescence High-Performance Liquid Chromatographic Analysis of Warfarin and Its Metabolites in Plasma and Urine

CHRISTOPHER BANFIELD and MALCOLM ROWLAND ˣ

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Abstract □ A stereospecific assay for the simultaneous determination of the enantiomers of warfarin and its major metabolites, 6- and 7-hydroxywarfarin and warfarin alcohols, in plasma and urine was developed. Involved in this determination was the formation of diastereoisomeric esters with carbobenzyloxy-L-proline, separation by normal-phase high-performance liquid chromatography, and detection by fluorescence after postcolumn aminolysis with *n*-butylamine. The determination limit for any enantiomer is in the order of 50–100 ng. The method was applied to the analysis of the enantiomers of warfarin and metabolites in plasma and urine of human subjects receiving racemic drug. The results for warfarin enantiomers are comparable with those obtained by an MS method, involving administration of a synthetic pseudoracemate [$^{12}C(R)$,$^{13}C(S)$]warfarin. In addition to all known metabolites, the detection of 7-*R*-hydroxywarfarin indicates that 7-hydroxylation is stereoselective rather than stereospecific.

Keyphrases □ Fluorescence, HPLC—stereospecific analysis of warfarin and its metabolites □ Warfarin—stereospecific fluorescence HPLC, metabolites □ Isomers—stereospecific fluorescence HPLC, warfarin and its metabolites

Warfarin [3-(α-acetonylbenzyl)-4-hydroxycoumarin] contains an asymmetric center. In humans, the *R*- and *S*-isomers show differences in pharmacological activity (1), hepatic clearance, and metabolism (2–4). Drugs have been shown to interact differently with the isomers; for example, when coadministered with phenylbutazone, the hepatic clearance

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