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In Vitro Cutaneous Metabolism of DDT in Human and Animal Skins

D. Moir, T. M. Marwood, R. P. Moody

Environmental and Occupational Toxicology Division, Bureau of Chemical Hazards, Health Protection Branch, Health and Welfare Canada, Tunney's Pasture, Ottawa, Ontario, K1A 0L2, Canada

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Interest in developing methods to assess human exposure to pesticides has led to a search for a suitable animal model for dermal absorption studies (Moody and Ritter, 1992). One of the pesticides of interest in dermal absorption research has been 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (DDT). As skin metabolism can have an important impact on dermal absorption (Kao *et al.*, 1989), any biotransformation of DDT as it passes through skin may be significant.

DDT can be ultimately metabolized to 2,2-bis(4-chlorophenyl) acetic acid (DDA) in mammalian systems; this metabolism begins with one of two steps, either to 2,2-bis(4-chlorophenyl)-1,1-dichloroethylene (DDE) or 2,2-bis(4-chlorophenyl)-1,1-dichloroethane (DDD) (Miyamoto *et al.*, 1988). Mammalian skin is capable of many metabolic processes similar to those of other organs (Pannatier *et al.*, 1978); therefore it is feasible that DDT, when applied to the skin would be metabolized to various derivatives and ultimately to DDA.

In the present study, a variety of skin types were incorporated into an *in vitro* investigation of the dermal metabolism of DDT. Human, rat, pig, and guinea pig skins were used along with a living skin equivalent, 'Testskin' (Organogenesis, Cambridge, MA). A method was developed to analyze for metabolites using high performance liquid chromatography (HPLC).

MATERIALS AND METHODS

^{14}C-Ring-UL-labelled DDT, specific activity 12.0 mCi/mM, (>95% radiochemical purity) was purchased from Sigma Chemical Co., St. Louis, Mo. HPLC analysis of this material showed the presence of labelled DDA at about 1%. DDD at about 0.4% and two unknowns at about 0.4% as well. A standard of p,p'-DDA was purchased from Caledon Laboratories, Georgetown, Ontario. Unlabelled standards of p,p'-DDT, DDD, and DDE were obtained from PolyScience Corp., Niles, IL. Glass-distilled HPLC-grade solvents were obtained from Caledon Laboratories, Georgetown, Ontario. Anhydrous sodium sulfate was purchased from Fisher Scientific, Fair Lawn, NJ.

Correspondence to: D. Moir

Female hairless guinea pigs and male Sprague-Dawley rats were purchased from Charles River Laboratories, Montreal, Quebec. Testskin is a patented living skin equivalent cultured by Organogenesis, Cambridge, MA. Yorkshire pig skins were obtained as a courtesy from the National Research Council, Ottawa, Ontario. Two samples of human skin were obtained from female subjects undergoing a panniculectomy at the Ottawa General Hospital, Ottawa, Ontario.

Rat skins were shaved before use. Skin sections of 500 μm thickness (except Testskin which was used at its original thickness of 300 μm) were obtained using a Padgett dermatome (Padgett Instruments, Kansas City Mo), washed with soap and water and punched into 2.0 cm^2 discs. These were mounted on Bronaugh flow-through diffusion cells (Crown Class, Somerville, NJ) leaving an exposed surface of 0.64 cm^2. The receptor fluid was HEPES-buffered Hank's balanced salt solution (HHBSS) containing gentamicin (50 mg/L) and 4% bovine serum albumin (BSA) filtered through 0.2 μm pore-size cellulose acetate filters (Nalgene Co., Rochester, NY) and aerated with 99% oxygen throughout the experiment. A water bath maintained the cells at 36.5°C. ^{14}C-labelled DDT was applied to the skins in acetone solution (50 μL, 0.5 μCi). Aliquots of receiver solution were collected every two hours over a period of 48 hours and stored at -10 °C until analysis. At 24 hours, the Testskin, guinea pig and the first four human skins were washed three times with soapy water and were then rinsed three times with distilled water. In all other experiments, washing was omitted as it was apparent that more DDT would be needed on the skin to produce detectable amounts of metabolites. In all experiments except the Testskin, the skin was kept after 48 hours in 5mL ethyl acetate, at -10 °C until analysis.

Initially, for analysis of the receiver solutions, groups of the two hour receiver solution fractions were pooled in order to ensure that metabolite concentrations would be large enough to be detected. In the Testskin experiment, all fractions were pooled and only one HPLC analysis was done for each skin. For the guinea pig experiment, two analyses were done per skin, the 0-24 and 24-48 hour fractions. All other experiments were divided into twelve hour fractions and four analyses done per skin.

Samples were extracted twice with two volumes of ethyl acetate by vortexing for 10 seconds followed by centrifuging for 15 minutes at 3000 rpm; the extracts were combined and reduced to dryness with gentle heating under a stream of nitrogen. The residue was dissolved in acetone, filtered and concentrated to a volume of about 50 μL. The ethyl acetate from the saved skin samples was washed with distilled water and dried over anhydrous sodium sulfate. Following this, the samples were reduced to dryness redissolved in acetone, filtered and reduced in volume to about 50 μL.

Standards were run between every four samples to ensure that retention times were consistent. Samples from the HPLC were collected in 3 minute aliquots for secondary analysis by scintillation counting (Results not shown).

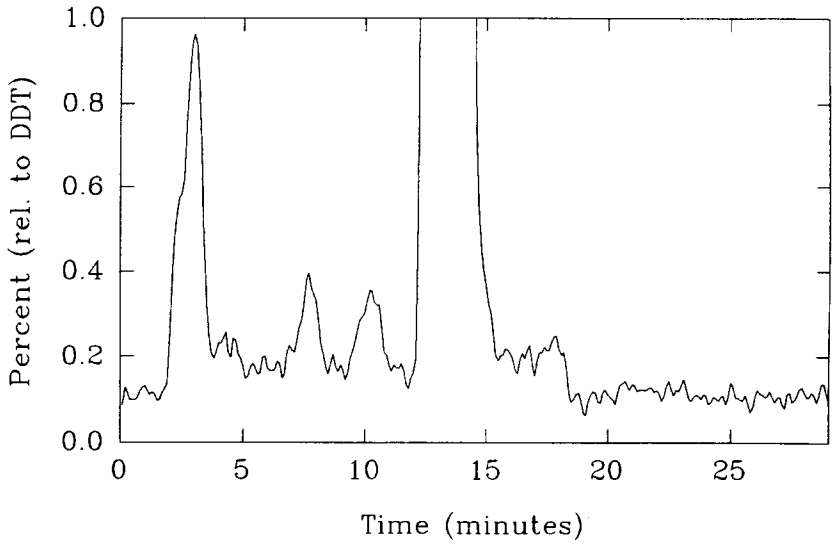

Figure 1. Labelled DDT standard analyzed using Berthold LB 506 C-1 Radioactivity Detector

The HPLC consisted of a Shimadzu SCL-6B System Controller, a SIL-6B Autoinjector and two LC-6A pumps along with a Waters Lambda-Max model 481 UV detector set at 235 nm and a Berthold LB 506 C-1 radioactivity monitor equipped with an yttrium glass 600 μL solid scintillator cell. The column was a Waters μBondapak C-18 (3.9 x 300 mm), 10 μ particle size and was protected by a Whatman C-18 guard column. The flow rate was 1.0 mL/min. and the mobile phase was a mixture of acetonitrile and water: 76:24 acetonitrile:water over the first 20 minutes then a linear change to 100 % acetonitrile over 5 minutes. The composition was returned to the original 76:24 and the column was equilibrated for 10 minutes. The UV detector was used to determine the retention times of the cold standards.

RESULTS AND DISCUSSION

No evidence of significant dermal metabolism was seen for any of the skins tested. The analysis of the receiver solutions, which were pooled for periods as long as 24 hours showed only DDT; the concentration of DDT in these solutions was very low, indicating a limited penetration through the skin. Testskin allowed 1.1% of the applied dose to penetrate into the receiver solution; all other skins allowed less than 1% with human skin permitting only 0.1%.

When our labelled DDT was subjected to analysis by our HPLC method, the presence of low level impurities was revealed. The chromatogram is shown in

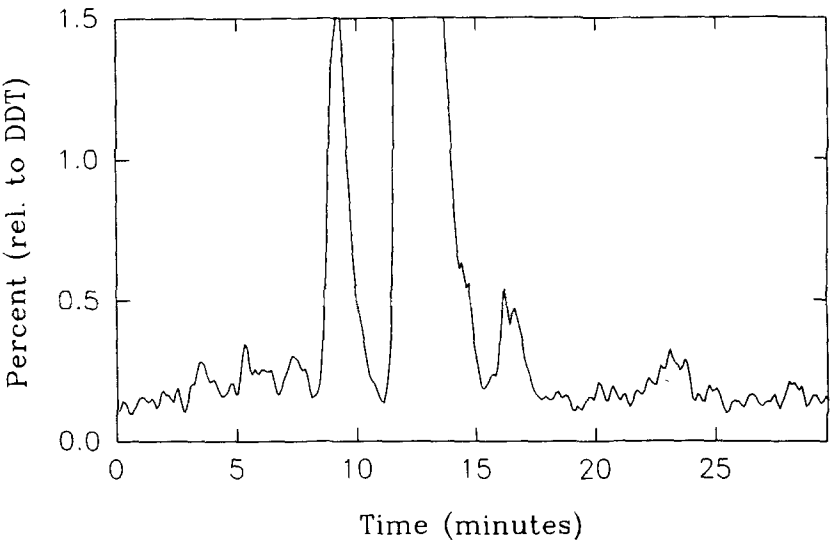

Figure 2. Extract of pig skin; the skin was stored at -10°C in 5 mL of ethyl acetate for 6 weeks.

Figure 1 and displays peaks at the retention times for DDA (3 minutes) DDD (10 minutes) and DDT (12.6 minutes) along with unknown peaks at 7.7 and 18 minutes. A very slight shoulder on the DDT peak might correspond to DDE.

The ethyl acetate in which the skins were stored served to extract the radiolabel quite efficiently as shown by the recovery of 22% of the applied dose from two guinea pig skins soaked in ethyl acetate and 17% of the dose found in guinea pig skin digest. These skins had been washed to remove surface DDT. Chromatograms of these extracts showed traces similar to that for the starting material. Little if any DDA was seen in these extracts, most likely as a result of its lower solubility in the ethyl acetate. Qualitatively, there was an increase in the size of the DDD peak in three of the six pig skins, to a value slightly over 1.5% of the DDT peak. One of these chromatograms is shown Figure 2. This was the greatest change detected and this was so slight that it is questionable whether or not this change arose from metabolism of DDT.

This HPLC method with radio detection is quite sensitive when there is enough labelled material to analyze. When looking at the extracts from skin dosed with half a μCi, it is possible to reproducibly detect peaks as small as 0.3% the size of the DDT peak. However, because of the poor absorption of DDT into the receiver solution, only DDT was detectable even in samples pooled for 24 hours.; any metabolite would have to be present in very high concentration to be detected.

In previous *in vitro* studies using thin-layer chromatography techniques and fuzzy

rat skin, percutaneous metabolism of DDT was not detected (Bronaugh *et al.*, 1990; Bronaugh *et al.*, 1989). The results did suggest that most DDT remained within the skin at the end of the experiment and that if metabolites were formed, they were either in undetectable amounts or retained within the skin. In agreement with these previous studies, no metabolites were found to have penetrated the skins.

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