Comparative in Vitro–in Vivo Percutaneous Absorption of the Pesticide Propoxur

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In vitro and in vivo skin absorption of the pesticide propoxur (2-isopropoxyphenyl N-methyl carbamate, commercially Baygon™ and Unden™, log Po/w 1.56, MW 209.2) was investigated. In vivo studies were performed in rats and human volunteers, applying the test compound to the dorsal skin and the volar aspect of the forearm, respectively. In vitro experiments were carried out in static diffusion cells using viable full-thickness skin membranes (rat and human), non-viable epidermal membranes (rat and human) and a perfused-pig-ear model. Percutaneous penetration of propoxur in human volunteers was measured by analysis of its metabolite (2-isopropoxyphenol) in blood and urine; in all other studies radiolabeled propoxur ([ring-U-14C]propoxur) was used. In order to allow for direct comparison, experimental conditions were standardized with respect to dose (150 μg propoxur per cm²), vehicle (60% aqueous ethanol) and exposure time (4 h). In human volunteers, it was found that approximately 6% of the applied dose was excreted via the urine after 24 h, while the potential absorbed dose (amount applied minus amount washed off) was 23 μg/cm². In rats these values were 21% and 88 μg/cm², respectively. Data obtained in vitro were almost always higher than those obtained in human volunteers. The most accurate in vitro prediction of the human in vivo percutaneous absorption of propoxur was obtained on the basis of the potential absorbed dose. The absorbed dose and the maximal flux in viable full-thickness skin membranes correlated reasonably well with the human in vivo situation (maximal overestimation by a factor of 3). Epidermal membranes overestimated the human in vivo data up to a factor of 8, but the species-differences observed in vivo were reflected correctly in this model. The data generated in the perfused-pig-ear model were generally intermediate between viable skin membranes and epidermal membranes.

Key Words: in vitro/in vivo comparisons; perfused-pig-ear model; viable skin and epidermal membranes; propoxur; rat; human.

It has been widely recognized that the skin can be an important route of entry for industrial chemicals and pesticides (e.g., Benford et al., 1999). For this reason, data on skin absorption are often required for risk assessment purposes and both animal models and in vitro systems are used to predict skin absorption in man. For the in vivo assessment of percutaneous absorption, guidelines have been drafted by the OECD (1996) and by the U.S. EPA (1996). Methodology for in vitro skin absorption studies has been described by various organizations, such as OECD (1996), U.S. EPA (1999), ECETOC (1993), ECVAM (1996), and COLIPA (Diembeck et al., 1999), but no generally accepted protocol has been developed.

Although the literature on in vitro percutaneous absorption is extensive, only a very limited number of studies has been designed for actual in vitro–in vivo comparisons (e.g., Beck et al., 1994; Dick et al., 1997; Ng et al., 1992). In most other studies, however, in vitro data are compared to in vivo data that were obtained under different experimental circumstances. This approach can easily lead to wrong conclusions since experimental variables such as dose, vehicle, humidity, temperature, anatomical site, and exposure duration have a considerable influence on the percutaneous absorption of compounds (ECETOC, 1993). Moreover, previously published studies compare in vivo and in vitro data on the basis of the cumulative amount of the test substance excreted (in vivo) or reaching the receptor fluid (in vitro), providing only partial insight in the more complex kinetic events of skin absorption.

The studies presented here were initiated with the aim of getting further insight into the relations between in vitro and in vivo measurements and into the predictive value of the in vivo rat model for the human situation. Experimental conditions were standardized with respect to dose, vehicle, and exposure duration. As a model compound, the pesticide propoxur (2-isopropoxyphenyl N-methyl carbamate, Baygon™, Unden™, log Po/w 1.56, MW 209.2) was selected based on its widespread use in outdoor agriculture and greenhouses, against a number of household and domestic pests. Moreover, handling of propoxur has been shown to result in extensive dermal exposure (Brouwer et al., 1993). In vivo studies were performed in rats and human volunteers, while in vitro experiments were carried out in static diffusion cells using viable skin membranes (rat and human), non-viable epidermal membranes (rat and human), and a perfused pig-ear model. In vivo and in
vitro absorption was compared on the basis of the absorbed dose after 4 and 24 h, the maximal flux, the lag time, and the potential absorbed dose.

MATERIALS AND METHODS

Chemicals

Propoxur (99.6% purity) was purchased from the Institute of Organic Industrial Chemistry, Warsaw, Poland. [Ring-\textsuperscript{14}C]propoxur (specific activity: 40.7 MBq/mmol) was synthesized at TNO Prins Maurits Laboratory (Rijswijk, the Netherlands). 2-Isoproxyphenol (IPP) was obtained from Aldrich Chemical Co. (Milwaukee, WI).

General Study Design

In all experiments, dose (150 µg propoxur per cm\textsuperscript{2}), vehicle (60% aqueous ethanol) and exposure time (4 h) were kept constant. For in vitro studies, rat in vivo studies, and experiments in the perfused pig ear, dosing solutions were prepared by mixing non-labeled propoxur with [ring-\textsuperscript{14}C]propoxur in order to reach the final concentration. The dose solutions for the human-volunteer studies consisted of non-radiolabeled propoxur only. After the exposure time of 4 h, the test compound was removed from the application site with cotton swabs. Samples were collected for a total of 6 h (perfused pig ear), 24 h (in vitro skin membranes), −48 h (human volunteers), and 72 h (rat studies).

In Vitro Experiments with Human and Rat Skin Membranes

In vitro experiments were performed according to the method described by Van de Sandt et al. (1993). In brief, sterile glass rings (internal area of 0.64 cm\textsuperscript{2}) were glued to the membranes using cyanoacrylate-based glue. The membranes were then carefully transferred into 6-well plates (Corning Costar, Cambridge, MA) on a Netwell insert (200-mm mesh Corning Costar, Cambridge, MA), which allows contact of the receptor fluid to the basal membrane of the epidermis, while the stratum corneum remains exposed to the air. The 6-well plates were placed in a humidified incubator gassed with 5% CO\textsubscript{2}, 40% O\textsubscript{2}, and 55% N\textsubscript{2} at 32°C. To obtain a homogeneous distribution of the receptor fluid the 6-well plates were rocked on a platform −9 times per min. The receptor fluid of the viable skin consisted of culture medium containing 10% fetal bovine serum, while the receptor fluid for the epidermal membranes consisted of saline (0.9% NaCl w/v) containing 0.01% sodium azide and 3% bovine serum albumin. In all experiments the volume of the receptor fluid was 1.2 ml.

Human skin was obtained directly after abdominal surgery at the Academic Hospital Utrecht (the Netherlands). The donors were young Caucasian females. The use of human skin for in vitro studies was approved by the local Medical Ethics Committee (TNO-MEC). Rat skin for viable skin membranes, rat in vivo studies, and experiments in the animals were transferred to the metabolism cages (Techniplast\textsuperscript{®}). Viable skin membranes had a thickness of approximately 1.0 mm. For epidermal membranes, rat skin was obtained from 26-day-old male Sprague-Dawley rats. For the preparation of both viable skin membranes and epidermal membranes, skin from the dorsal side of the animals was used. Epidermal membranes were prepared by immersion of the skin in water heated to about 60°C (human epidermis) or by overnight immersion of the skin in 2-M NaBr (rat epidermis).

Prior to the application of propoxur to the skin membranes, the integrity of the membranes was assessed on the basis of the penetration of [\textsuperscript{3}H]-labeled water over a 3-h period. On the basis of our historical database and reported data (e.g., Barber et al., 1992), only membranes with a K\textsubscript{p} value of less than 2.0 × 10\textsuperscript{−3} cm/h (human) or 3.5 × 10\textsuperscript{−3} cm/h (rat) were included in the experiments. In order to assess the membrane integrity during the entire experimental period, testosterone ([4-\textsuperscript{14}C]testosterone mixed with non-radiolabeled testosterone, 200 µg/cm\textsuperscript{2} in ethanol/water (60/40)) was used as a reference compound in each experiment. Sampling of the receptor fluid and calculation of the percutaneous absorption of this reference compound were identical for propoxur (see below). In all experiments, the penetration rate of testosterone was within the range of historical data of our laboratory.

The dose solution consisted of [ring-\textsuperscript{14}C]propoxur mixed with non-labeled propoxur in ethanol/water (60/40), of which 50 µl was applied in the glass rings. After an exposure period of 4 h, the remaining test compound was removed from the application site with cotton swabs. Samples of receptor fluid (200 µl) were collected at various time points after application until 24 h post-application. Directly after each sampling the original volume of the receptor fluid was restored by adding 200 µl fresh receptor fluid to each well. In order to determine the recovery at the end of the experiment, the skin membranes were digested in 5 ml 1.5 M KOH in 20% ethanol, the remaining receptor fluid was collected, and the wells were washed ×2 with 1.0 ml ethanol.

The Perfused Pig-Ear Model

Pig ears were prepared and perfused with whole-pig blood as described by de Lange et al. (1992, 1994). Briefly, on arrival in the laboratory, ears of healthy domestic pigs (75–100 kg) were cannulated with polyethylene cannulae in the vena auricularis intermedia and the arteria auricularis lateralis. Thereafter, the ears were kept in a temperature- and humidity-controlled environment (30°C, 40–60% relative humidity) and perfused with phosphate-buffered saline, containing glucose and heparin. Leakages along the cutting edge were coagulated or glued. After an equilibration period of 15 min, perfusion with autologous oxygenated and filtered whole blood was started. In all experiments, the perfusion pressure and the perfusion flow were carefully monitored during the 6-h perfusion. The mean values of the experiments were between 34 and 75 mm Hg and between 0.42 and 0.48 ml/min, respectively. As a parameter of cell viability, glucose consumption was measured at various times and was between 401 and 565 µg/min/100 g. At the end of the permeation experiments, the preparations were weighed and compared to pre-perfusion weights, in order to establish edema formation as an indication of tissue viability. Results obtained using a particular ear were discarded if one of the above parameters did not fall within previously established limits. A glass ring (area 10 cm\textsuperscript{2}) was glued onto the ear by means of cyanoacrylate glue.

The dose solution consisted of [ring-\textsuperscript{14}C]propoxur mixed with non-labeled propoxur in ethanol/water (60/40). After 15 min of perfusion with blood, 200 µl of the dose solution was applied to the skin in each glass ring, covering the complete application area, and the rings were covered with a watch glass for the period of the experiment. After 4 h, remaining test compound was removed by cleaning the exposed area with cotton wool. At the end of the experiment (6-h post application), a 0.9-cm diameter punch biopsy was taken through the ear and the skin above and below the cartilage along with the cartilage was dissolved separately in Soluene\textsuperscript{®}.

Rat in Vivo Experiments

Experiments were performed with male Wistar rats (Crl:WI\textsuperscript{®} BR)) of 280 ± 12 g. After cannulation of the vena jugularis, the rats were acclimatized to the laboratory conditions for 3 days. During the experiment, the animals were housed individually in metabolism cages. Relative humidity was between 35 and 45%, while the temperature was between 20.5 and 22.0°C.

Dermal administration. The day prior to the experiment, the hair from the backs and flanks of the animals was removed using an electric clipper, and the animals were transferred to the metabolism cages (Techniplast\textsuperscript{®}). Prior to the administration of the dose solution, a non-absorbing “O-ring” (Erik's BV, Alkmaar, the Netherlands) with an area of 10 cm\textsuperscript{2} was glued to the back of the rat with cyanoacrylate adhesive. The dose solution consisted of [ring-\textsuperscript{14}C]propoxur mixed with non-labeled propoxur in ethanol/water (60/40). The dose solution (100 µl per animal) was applied and spread evenly within the O-ring. To achieve semi-occlusive exposure conditions, the application area was covered with Tensoplastic elastic adhesive bandage (Smyth and Nephew Medical Limited, Hull, UK) approximately 5 min after dosing and kept in
place for the remainder of the exposure period, avoiding contact between dressing and skin at the site of application. Five small perforations were made in the bandage above the application area. For exposure under occlusion, the O-ring was covered with laboratory film (Parafilm “M”, American National Can Company, Greenwich, CT) prior to the application of the non-occlusive dressing. After the exposure period of 4 h, the dressing was removed and the site of application was thoroughly cleaned 10 times with cotton swabs. After cleaning the application site, a fresh, non-occlusive cover (without additional perforations) was applied in order to prevent contact with the exposed skin.

**Intravenous administration.** In order to determine the T1/2 value and to allow for the determination of the absorption rate, 4 animals each received a 500 μl intravenous bolus dose of [ring-14C]propoxur (0.15 MBq/ml) by injection into the tail vein (1.38 mg propoxur per kg body weight).

**Sample collection.** Urine and feces was collected at various intervals after administration. Blood samples (approximately 100 μl) were collected via the cannula. At the end of the experiment, the cotton swabs, the dressings, and the O-ring were extracted in ethanol/water (60/40). The skin at the site of application, a non-treated skin area, and the residual carcass were collected separately and digested with 1.5 M KOH in 20% ethanol. All cages were washed thoroughly with water/ethanol/triton X-100 (50/50/1 [v/v/v]).

**Human Volunteer Study**

Human volunteer studies were performed according to Meuling et al. (1996). Three human volunteers (males, 23–24 years, 68–88 kg body weights) were selected after a medical screening, which included a health questionnaire and a physical examination of the application sites. Initially, the kinetic behavior of propoxur was determined after intravenous administration. Five to seven days after the iv experiment, the same human volunteers were dosed dermally with propoxur. The study was conducted according to ICH Guidelines of Good Clinical Practice and was approved by the local Medical Ethics Committee (MEC-TNO).

**Dermal application.** The solution consisted of non-radiolabeled propoxur (50 mg/ml) in water/ethanol (40/60% v/v), of which 0.3 ml was evenly applied onto an area of 100 cm² of the volar aspect of one forearm of each subject. After the 4-h exposure period, the excess of the applied substance was washed off by wiping with dental cotton-wool rolls. During the exposure, the subjects were housed in a cabin and had their forearms placed in an incubator. Both cabin and incubator were controlled for temperature (25 ± 2°C) and relative humidity (50 ± 10%).

**Intravenous dosing.** The individual kinetics of propoxur in the 3 subjects was established by dosing 3.5 mg propoxur in an ethanol-saline solution, prepared according to good manufacture practice (GMP). This solution was administered intravenously through infusion during ~40 min, at a constant rate of about 6 ml per min, into a cubital vein.

**Sample collection.** For each volunteer, baseline urine values of IPP were established prior to the start of every experiment. During the experiments, all produced urine was collected every 3 h during the first 16 h (until bedtime). On subsequent days, after arising the next morning, all produced urine was collected and every 4 h up to about 48 h post-dosing. Blood sampling was performed pre-exposure and frequently at pre-determined time intervals during exposure, via a cannula inserted in the contralateral arm in the intravenous dosing experiment, and in both dermal application experiments. On subsequent days, blood samplings were performed at about 24 and 48 h post-dosing, via single venipuncture.

**Radioactivity Measurements**

Radioactivity in the samples of the in vitro experiments, the in vivo rat study, and the perfused-pig ear experiments was determined by liquid scintillation counting (LSC) using DOT-DPM™ (digital overlay technique, using the spectrum library and the external standard spectrum) for quench correction. Radioactivity in feces and blood was determined by combustion analysis, using a Packard sample Oxidizer System 387. The CO₂ formed was absorbed in Carbosorb™ and mixed with scintillation fluid.

**Chemical Analysis of Non-radiolabeled Propoxur and 2-Isopropoxyphenol**

In the human volunteer study, all materials used for dermal and intravenous administration and removal after dermal exposure (i.e., pipette tips, syringes, and cotton rolls) were analyzed for propoxur. Analysis was performed by reversed-phase HPLC with fluorescence detection. In the plasma and urine samples, the primary metabolite of propoxur, 2-isopropoxyphenol (IPP), was analyzed. No intact propoxur was found in these samples. IPP was determined by capillary gas chromatography and mass-selective detection (Leeneers et al., 1992), after the samples were boiled with hydrochloric acid in order to hydrolyze conjugated IPP and extracted with hexane.

**Calculations**

**Absorbed dose [μg/cm²].** These amounts were calculated over 4 and 24 h as the total amount of propoxur equivalents excreted in the urine and feces (in vivo rat), or the total amount of propoxur equivalents reaching the receptor fluid or perfuse (in vitro). In human volunteers, the absorbed dose was calculated by dividing the amount of IPP excreted in the urine after dermal exposure by the amount of IPP excreted in the urine after intravenous dosing.

**Percutaneous absorption rate in vivo [μg/cm²/h].** This rate was determined using the concentration time course of propoxur equivalents in blood after dermal exposure, and the time course after a reference intravenous dosing. From the latter time course, a function describing the individual kinetics was calculated using linear-system dynamics (Opdam, 1991), and expressed as the sum of 2 exponential terms. This function was used in the point-area-deconvolution method described by Vaughan and Dennis (1978) to calculate the percutaneous-absorption-rate time course from the blood concentrations after dermal dosing (Opdam et al., 1995; Fishet et al., 1985).

**Maximal flux [μg/cm²/h].** The maximal flux was calculated from the linear portion of the cumulative penetration curve.

**Permeability coefficient.** This Kp value [cm/h] for [1H]H₂O was calculated according to ECETOC (1993), by dividing the maximal flux [μg/cm²/h] by the applied dose.

**Lag time [h].** Lag time was obtained by extrapolating the linear portion of the cumulative penetration curve to the x-axis.

**Potential absorbed dose (PAD).** The PAD was defined by the applied dose minus the amount washed off the application site after the 4-h exposure period.

**Factor of difference (FOD).** The FOD was defined by dividing the mean value obtained in the rat or in an in vitro model by the mean value obtained in human volunteers. For example, for in vitro-in vivo comparison of fluxes: FOD = human flux / in vitro human flux in vivo.

**RESULTS**

**In vivo and in vitro skin absorption of propoxur was compared on the basis of several endpoints: the absorbed dose after 4 and 24 h, the maximal flux, the lag time, and the potential absorbed dose (summarized in Table 1).**

**In Vitro Experiments with Human and Rat Skin Membranes**

**Viable skin membranes.** The amount of propoxur equivalents reaching the receptor fluid through viable rat skin was 1.1 ± 0.3% and 7.3 ± 1.4% of the dose after 4 and 24 h, while for viable human skin, these percentages were 1.7 ± 0.5% and 9.7 ± 2.3% (Fig. 1). The maximal flux was 1.0 ± 0.3 μg/cm²/h (rat skin) and 1.7 ± 0.4 μg/cm²/h (human skin). The potential absorbed dose after a 4-h exposure in rat skin was 28.8 ± 7.6
mu/g/cm^2, while in human skin the potential absorbed dose was 16.9 ± 8.1 mu/g/cm^2. The recovery of the radioactivity was 100.0 ± 6.2% (from human skin) and 92.1 ± 3.7% (from rat skin).

Epidermal membranes. The amount of propoxur equivalents reaching the receptor fluid through rat epidermal membranes was 13.5 ± 1.8 and 30.3 ± 4.5% of the dose after 4 and 24 h, respectively, while for human epidermal membranes these percentages were 9.5 ± 3.8% and 25.1 ± 6.7% (Fig. 1). The maximal flux was 7.2 ± 1.0 mu/g/cm^2/h (rat epidermis) and 4.9 ± 1.5 mu/g/cm^2/h (human epidermis). The potential absorbed dose in rat epidermal membranes was 54.0 ± 9.0 mu/g/cm^2, while in human epidermal membranes the potential absorbed dose was 58.8 ± 6.0 mu/g/cm^2. The recovery of the radioactivity was 107.9 ± 1.6% (human epidermis) and 102.3 ± 1.6% (rat epidermis).

The Perfused-Pig-Ear Model

The amount of propoxur equivalents reaching the perfusate fluid was 5.3 ± 3.2% and 7.6 ± 4.5% of the dose after 4 and 6 h (Fig. 2). The maximal flux was 2.5 ± 1.3 mu/g/cm^2/h and the potential absorbed dose was 26.1 ± 6.6 mu/g/cm^2. The recovery of the radioactivity was 94.7 ± 0.5%.

Rat in Vivo Experiments

When propoxur was applied topically under semi-occlusive conditions, 5.1 ± 0.9%, 13.2 ± 0.8%, and 15.1 ± 0.8% of the applied dose was excreted via the urine after 4, 24, and 72 h (Fig. 3A), while under occlusion these percentages were 16.6 ± 9.0%, 31.2 ± 6.0%, and 34.1 ± 6.1% (Fig. 3B). The amount propoxur equivalents present in the feces remained below 1% of the dose in both groups. The potential absorbed dose, defined as the amount applied minus the amount present...
in the ring, cover, and skin wash was 48.3 ± 2.9 μg/cm² (semi-occlusive application) and 87.8 ± 3.7 μg/cm² (semi-occlusive conditions).

After intravenous administration, the maximal concentration of propoxur equivalents was 1122 ng/ml blood and the half-life was 30 min. After semi-occlusive dermal administration, the peak concentration of 151 ng/ml was reached after 30 min. Under occlusion, the peak concentration of 356 ng/g was reached after 60 min. Using the blood kinetics of the intravenous dosed rats, percutaneous absorption rates were calculated. It was observed that the maximal flux was ca. 2.4 times lower when exposure was under semi-occluded compared to occluded circumstances: 8.7 ± 0.8 versus 20.9 ± 4.4 μg/cm²/h.

At the end of the experiment (72 h post-application), the recovery of the radioactivity was determined. After dermal exposure, the total amount in urine, feces, cage-wash, carcass, control skin and blood samples was 15.7 ± 0.8% and 36.1 ± 6.1% under semi-occlusive and occlusive exposure conditions, respectively. The amount remaining in the skin was 5.0 ± 0.2% (semi-occlusive) and 11.2 ± 2.1% (occlusive) of the applied dose. The recovery of radioactivity was 90.1 ± 1.9%, 90.6 ± 2.5% and 95.5 ± 1.5% after semi-occlusive dermal exposure, occlusive dermal exposure, and intravenous exposure, respectively.

Human Volunteer Study

The percutaneous absorption of propoxur was assessed in 3 individuals (Figs. 4A and 4B). The mean absorbed dose of propoxur (measured as IPP) was 0.5 ± 0.5% and 3.7 ± 2.9% after 4 and 24 h, respectively. The potential absorbed dose after the 4-h exposure was 23.0 ± 9.0 μg/cm². After intravenous
administration, the maximal concentration IPP in the blood was 529.9 ± 442.1 ng/ml and the half time was 146 min. After non-occlusive dermal administration, the peak concentration of 10.1 ± 7.5 ng/ml was reached after 440 min. Using the blood kinetics after intravenous administration, percutaneous absorption rates were calculated. The maximal flux was 1.0 ± 1.1 μg/cm²/h.

DISCUSSION

At present, skin penetration studies for risk evaluation purposes are most often performed using animal models. In addition, in vitro methodology becomes increasingly popular for the assessment of skin penetration of agro-chemicals, pharmaceuticals, and cosmetic ingredients. We here report a comparative study on the percutaneous absorption of a model compound (propoxur) in rats, human volunteers, and several in vitro systems. Although percutaneous absorption of propoxur has been reported previously (Feldmann and Maibach, 1974; Meuling et al., 1997; Van de Sandt et al., 1993), the present study was specially designed to compare in vitro and in vivo methodology under identical experimental conditions.

In Vivo–in Vitro Comparison

When determining the predictive value of in vitro systems for human in vivo percutaneous absorption, it is important to realize that both the in vitro and the in vivo data have a certain variation. In fact, quite large differences in barrier function between human individuals have been reported (e.g., Schaefer and Redelmeier, 1996), and also in the present study we found a relatively high variation among the human volunteers. In addition to inter-individual differences, considerable regional differences exist in the barrier function. Extremely high penetration rates are known to occur through the skin of genitalia, for example, but limited differences exist between human skin from arm and abdomen (Lotte et al., 1987) which were used in the present study for the in vivo and in vitro parts, respectively.

In the present study, in vivo and in vitro skin absorption of propoxur were compared on the basis of several endpoints: the absorbed dose after 4 and 24 h, the maximal flux, the lag time, and the potential absorbed dose. Based on the mean factor of difference (FOD) between the in vitro systems and the “golden standard” human volunteers (Fig. 5), it appeared that the values obtained in the in vitro models were almost always higher than those obtained in human volunteers. For the absorbed dose after 24 h, the in vitro models overestimated the human in vivo situation by a factor of 2 to 8, while the over-prediction was a factor of 1 to 7 on the basis of the maximal flux. The potential absorbed dose observed in vitro predicted the human in vivo situation the most accurately, with a maximal overestimation of a factor of 2.5.

Epidermal membranes were clearly more permeable for propoxur than viable skin membranes, while absorption of propoxur in the perfused-pig-ear model was intermediate between epidermal membranes and viable skin. This indicates that after penetration through the epidermis, propoxur is partially retained in the dermis and is only slowly released into the receptor fluid. Such a dermal depot in vitro has been reported before for lipophilic compounds (Moody and Nadeau, 1993; Schaefer and Redelmeier, 1996), while for hydrophilic compounds, no differences were observed between full-thickness skin and epidermal membranes (Clowes et al., 1994). In the present study, the reason for the retention in the dermis cannot be due to insolubility of propoxur in the receptor fluid, since the water solubility of propoxur is 1.9 mg/ml and only ~0.1 mg of propoxur was applied on each membrane (the volume of the receptor fluid being 1.2 ml). Therefore, the difference must be due to other factors, such as binding of (metabolites of) propoxur to dermal components. Since the epidermal membranes were the most over-predicting model, we must assume that in the in vivo situation, the dermis acts also as a partial depot for propoxur.

Cross-species Comparison in Vitro and in Vivo

In vivo absorption kinetics of propoxur differed considerably between rats and humans. Under semi-occluded exposure conditions, the rat overestimated the (non-occluded) human situation with a factor of 2 to 10, based on the absorbed dose, the maximal flux, and the potential absorbed dose. Moreover, peak concentrations in the blood of rats were observed 0.5 h after administration, after which the blood concentration decreased rapidly. In contrast, peak blood concentrations were reached in human volunteers ~7 h after administration. These data are in line with the general observation that rat skin is more perme-
able than human skin (ECETOC, 1993) which is likely caused by the different structure of the skin (e.g., number of appendages, intercellular lipid composition of the stratum corneum, and corneocyte surface area) (Schaef er and Redelmeier, 1996). It should be noted, however, that some cases have been reported where rat skin was found to be less permeable than human skin (e.g., Hotchkiss et al., 1992). In vitro, rat epidermal membranes were more permeable than human epidermal membranes, thereby reflecting the in vivo situation. In contrast, this species difference was not observed when using viable full-thickness skin membranes (with the exception of the potentially absorbed dose). This observation may be explained by the biotransformation capacity of full-thickness skin membranes. While it has been reported that propoxur was hydrolyzed and subsequently conjugated in viable skin membranes (Van de Sandt et al., 1993), propoxur was not metabolized in epidermal membranes. Since viable rat skin membranes metabolize propoxur to a larger extent than human skin (unpublished results), one could speculate that the metabolites of propoxur penetrate the dermis at a lower rate than the parent compound and/or bind to dermal components. In vivo this may be of less importance since the parent compound and its metabolites are taken up by the microcirculation in the upper dermis. The lower permeability of viable rat skin compared to viable human skin was limited to propoxur, since the penetration rate of testosterone (the reference compound used in vivo studies) was higher in the viable rat membranes when compared to viable human membranes.

Percutaneous absorption of propoxur in rats was clearly increased under occlusive conditions. These results in rats are in line with data obtained in human volunteers with the same compound (Meuling et al., 1991). It should be noted that animal studies couldn’t be performed completely non-occlusively, since the (gas-permeable) cover is necessary to protect the application site from coming into contact with the cage. Although the occurrence of occlusion can thus lead to increased skin absorption (which may have implications for the health risk assessment of, e.g., workers), it has been demonstrated that occlusion does not always enhance the percutaneous absorption of chemicals (Bucks et al., 1989).

Conclusions

The present study demonstrated that in vitro methods accurately predicted human in vivo percutaneous absorption of propoxur on the basis of the potential-absorbed dose. With respect to the other parameters studied here (absorbed dose and maximal flux), considerable differences were observed between the various in vitro models. In viable full-thickness skin membranes, these parameters correlated reasonably well with the human in vivo situation. Although epidermal membranes overestimated human in vivo data, the species-differences observed in vivo were reflected correctly in this model. The data generated in the perfused-pig-ear model were generally intermediate between viable skin membranes and epidermal membranes. Interestingly, the in vivo rat model clearly overestimated human skin absorption of propoxur. From a risk-assessment standpoint, the data obtained in the various in vitro models could be applied in the estimation of human risk, since the systems were reasonably predictive and always overestimated human in vivo absorption. It should be noted, however, that propoxur is a relatively hydrophilic compound. Therefore, further comparative studies must establish the predictive value of the various in vitro methods for lipophilic penetrants.

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