

PERCUTANEOUS ABSORPTION
OF
OCTOPIROX

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SUMMARY

Radioactive Octopirox, [^{14}C] Octopirox, supplied by Hoechst, was administered in aqueous polyethelene glycol 200 solution, to rats by oral intubation, intraperitoneal and subcutaneous injection and its route and rate of excretion was examined by radiotracer methods. The major excreted component was identified by thin layer chromatography as unchanged Octopirox.

For topical treatment, [^{14}C] Octopirox was dissolved in an anionic shampoo base and applied to rat skin. A number of variables were examined for their effect on skin penetration. These were the effect of rinsing, occlusion of the skin, the concentration of applied Octopirox, the duration of contact with the skin before rinsing and the presence or absence of hair.

When given by intubation or injection [^{14}C] Octopirox was excreted by the rat mostly in the faeces (65-85% of the dose) with smaller amounts (6-19%) in the urine. Most of the excretion occurred within 24 hours of dosing.

Skin penetration of Octopirox at 1% (w/v) in the shampoo without rinsing was $65.1 \mu\text{g}/\text{cm}^2$ under occlusive and $38.2 \mu\text{g}/\text{cm}^2$ under non-occlusive conditions. In a 'rinse off' treatment, penetration was reduced to $3.4 \mu\text{g}/\text{cm}^2$ under occlusive and $2.0 \mu\text{g}/\text{cm}^2$ under non-occlusive conditions.

There was a dependence of skin penetration of Octopirox on duration of contact up to 10 minutes after application. Penetration of 1% Octopirox increased from $2.4 \mu\text{g}/\text{cm}^2$ after 2.5 minutes exposure to $4.5 \mu\text{g}/\text{cm}^2$ after 10 minutes' duration of contact. The increases were statistically significant ($P = 0.05$). There was no further increase in penetration of Octopirox at 20 minutes application of 1% Octopirox in the shampoo.

Skin penetration and deposition of Octopirox were both proportional to Octopirox concentration between 0.1% (w/v) and 1% (w/v). Skin penetration increased from 0.31 to $3.6 \mu\text{g}/\text{cm}^2$ respectively. Skin deposition immediately after rinsing increased from 0.8 to $7.6 \mu\text{g}/\text{cm}^2$ for the ten-fold increase in Octopirox concentration.

There was no significant difference between the penetration through clipped skin and hairy skin from an application of 1% Octopirox for 5 minutes followed by rinsing. Under occlusive conditions, measured values were $2.8 \mu\text{g}/\text{cm}^2$ for clipped and $3.4 \mu\text{g}/\text{cm}^2$ for hairy skin. Under the non occlusive conditions, the measured value was $1.5 \mu\text{g}/\text{cm}^2$ for both types of skin.

Whether given by mouth, injection or application to the skin, Octopirox was excreted essentially unchanged.

When given by mouth (4.8 mg/kg body weight), most of the radioactivity was associated with the gastrointestinal tract. The liver contained up to 3 μg at 6 hours after intubation, whereas the kidneys contained up to 0.3 μg at 6 hours. Other tissues analysed contained only nanogram equivalents of Octopirox. Blood levels reached a maximum of 0.157 $\mu\text{g}/\text{ml}$ at 2 hours after intubation and declined to 0.007 $\mu\text{g}/\text{ml}$ at 48 hours.

When applied topically (15.4 mg/kg body weight), without rinsing and with occlusive protection of the skin, tissue levels were similar to those after oral intubation. Blood levels were greater, reaching 0.32 $\mu\text{g}/\text{ml}$ at 6 hours. However, when the skin was rinsed and protected with a non-occlusive patch, tissue levels were less than 10% of the unrinsed occluded animals and the blood levels were much reduced to a maximum of 0.018 $\mu\text{g}/\text{ml}$ at 1 hour after application and falling steadily thereafter.

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The safety factor estimated for the consumer using a shampoo containing 1% Octopirox is 18,500 so that the possibility of systemic effects due to absorption through the skin is remote.

QUALITY ASSURANCE STATEMENT

Environmental Safety Laboratory
Unilever Research
Colworth House
Sharnbrook
Beds.

Study Title:
Skin penetration studies on Octopirox.

Study No: A1 83.01

Inspection of the various phases of this study have been conducted by the Quality Assurance Unit. The dates on which inspections were made and the dates on which any findings were reported to the Study Director and to management are given below. This programme has been complemented by routine inspections of facilities within the Laboratory.

PHASE	DATE OF INSPECTION	DATE REPORTED
Protocol Audit	6/ 1/1983	6/ 1/1983
Procedural Inspection	11/ 1/1983	11/ 1/1983
Procedural Inspection	12/ 1/1983	14/ 1/1983
Procedural Inspection	14/ 1/1983	14/ 1/1983
Procedural Inspection	14/ 1/1983	14/ 1/1983
Procedural Inspection	16/ 2/1983	21/ 2/1983
Procedural Inspection	18/ 2/1983	21/ 2/1983
Study Report Audit	27/ 7/1983	8/ 8/1983

This report has been accepted by the QAU as being an accurate presentation of the raw data and findings of the study.

Signed



Quality Assurance Manager

Date

19th August 1983

QUALITY ASSURANCE STATEMENT

Environmental Safety Laboratory
Unilever Research
Colworth House
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Beds.

Study Title:
Skin penetration studies on Ocropirox from a shampoo base.

Study No: AI 82.06

Inspection of the various phases of this study have been conducted by the Quality Assurance Unit. The dates on which inspections were made and the dates on which any findings were reported to the Study Director and to Management are given below. This programme has been complemented by routine inspections of facilities within the Laboratory.

PHASE	DATE OF INSPECTION	DATE REPORTED
Protocol Audit	17/ 9/1982	20/ 9/1982
Procedural Inspection	11/10/1982	15/10/1982
Procedural Inspection	11/10/1982	15/10/1982
Procedural Inspection	15/10/1982	15/10/1982
Procedural Inspection	14/12/1982	21/12/1982
Procedural Inspection	14/12/1982	21/12/1982
Study Report Audit	26/ 7/1983	8/ 8/1983

This report has been accepted by the QAU as being an accurate presentation of the raw data and findings of the study.

Signed



Quality Assurance Manager

Date 18th August 1983.

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1. INTRODUCTION

Octopirox, supplied by Hoechst, is a substituted pyridine proposed for use as an anti-dandruff component in shampoos.

Some toxicological studies have been done. Hoechst themselves have investigated its chronic toxicity in a 90 day feeding study in rats and a teratology study in rabbits (Gilpin, 1981), as well as a study of turnover and skin penetration (Kellner and Eckert, 1980).

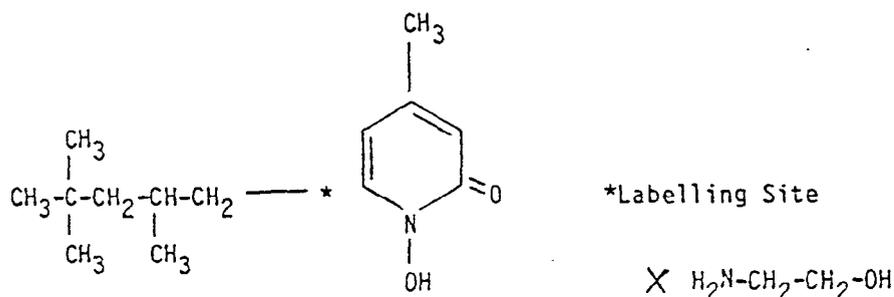
The purpose of this report is to present data on the rate and route of excretion in the rat of Octopirox given by intubation, injection and application to the skin. Blood levels after intubation and skin application have been measured and the nature of the end products of metabolism from different routes of administration have been investigated.

In the skin penetration studies, Octopirox has been applied to the skin in a shampoo which was rinsed away, as the consumer is expected to use the product. Factors affecting penetration, including duration of contact and applied concentration, have been studied. From the data, the expected human body burden has been calculated and an estimate made of the safety-in-use of Octopirox in shampoo products.

2. MATERIALS AND METHODS

2.1. [¹⁴C] Octopirox and carrier Octopirox

Octopirox [^{6-¹⁴C}] (I) was supplied by Hoechst AG, Germany. It was ring labelled and had a nominal specific activity of 17 $\mu\text{Ci} \times \text{mg}^{-1}$ (Hoechst ref. Lot 110671, ESL ref. S13246 T1).



Octopirox - (pyridone - 6 ¹⁴C) - ethanolamine salt.

Carrier Octopirox was obtained from URL Port Sunlight (ESL ref. S13225 T1) who in turn had obtained it from Hoechst.

2.2. Shampoo base - (C635 T1)

The shampoo base was a proprietary shampoo without Octopirox containing 16% of the detergent, sodium lauryl ether sulphate. It was diluted with water to 50% (w/w) immediately before use to reduce viscosity and facilitate accurate dispensing.

2.3. Preparation of test solutions of [¹⁴C] Octopirox

2.3.1. Turnover study

To 9.9 mg of [¹⁴C] Octopirox in a vial were added 2 drops of absolute ethanol from a Pasteur pipette and 8.5 ml of a 50% (v/v) distilled water solution of polyethylene glycol 200. The mixture was well shaken and warmed at 50°C in an oven for 5 to 8 minutes until it became clear.

Aliquots (0.5 ml) of the stock solution were diluted to 250 ml and 0.5 ml aliquots were counted to determine accurately the dose of [¹⁴C] Octopirox to the rats.

2.3.2. Skin penetration studies

For all these studies, [¹⁴C] Octopirox with or without carrier were weighed in a tared vial. To this was added a known volume of freshly diluted 50% (w/w) shampoo. The contents were warmed in the oven at 50°C until they became clear. Proportions of carrier varied depending upon the final concentration of Octopirox. Samples of the various test solutions were diluted to a known volume with water and aliquots of this were counted for ¹⁴C to determine the amount of radioactivity applied to the skin.

2.4. Animals and treatment

Colworth Wistar rats were used throughout the studies. They were housed in plastic metabolism cages for the separation and collection of urine and faeces and were fed Spital pelleted diet and water ad lib. All rats were housed in the cages for 24 hours before treatment to collect urine and faeces which served as experimental blanks in assays of radioactivity.

2.4.1. Turnover study

Six male and six female rats weighing about 130g were placed in separate metabolism cages and numbered sequentially. The cages were equipped with CO₂ absorbers, which were towers containing 30% ethanolamine in 2-methoxyethanol, through which the air from the cages was withdrawn. Four rats, two males and two females, were intubated with 0.5 ml (20.079 x 10⁶ dpm) of the test solution. Two male and two female rats were injected intraperitoneally and the remaining two male and two female rats were injected subcutaneously with 0.5 ml (20.079 x 10⁶ dpm) of the test solution. For injection, each rat was anaesthetised lightly with a mixture of cyclopropane, oxygen and carbon dioxide. After dosing, the rats were returned to their respective metabolism cages.

1 ml aliquots of CO₂ trap solutions were sampled hourly during the first six hours, then at 24 hours after dosing. CO₂ was not measured thereafter (the level of ¹⁴C was close to background radioactivity).

Urine was collected at 6 and 24 hours after dosing and then daily for four days.

Faeces were collected every 24 hours for four days.

At 96 hours after dosing, all rats were anaesthetised with cyclopropane. 2 ml of heart blood was collected from each rat, transferred to an anticoagulant vial, mixed well on a centrifugal mixer and stored in the refrigerator until assay of ^{14}C . The rats were then killed by cervical dislocation and the spleen, kidney, bladder and liver excised. These organs and the residual carcasses were assayed for radioactivity.

2.4.2. Skin penetration studies

For topical applications to rats, a standard treatment regime was adopted. The hair on the backs of all rats was clipped off 24 hours before treatment. The animals were housed in their individual cages. The separated urine and faeces collected during the 24 hours, were treated as excreta blanks. Prior to treatment, the rats were anaesthetised with cyclopropane, a 10 cm² area on the back was marked with a felt tipped pen. 200 ul of 50% (w/w) shampoo solution containing [^{14}C] Octopirox were applied to the skin and lightly massaged with the dispensing applicator for 1 minute to produce a lather. After a specified duration of contact, the skin was rinsed carefully over a collecting funnel with an excess of distilled water and the rinsing collected. The skin was then dried with warm air and covered with a protective patch and the animal replaced in its metabolism cage.

Two types of protective patch were used in this study. Of limited use was the non-occlusive protective patch described by Howes (1975). The other was an occlusive patch which was similar in construction but was lined with polythene film which was in contact with the treated skin and prevented any evaporation from the skin surface thus fully hydrating the skin. We normally expect maximum possible penetration under these circumstances.

Excreta were separated and collected at 24 and 48 hours. At 48 hours after application the animal was anaesthetised with cyclopropane, the protective patch removed and heart blood withdrawn. The animals were killed by cervical dislocation and the treated area of skin plus a 'border' of up to 2 cm was excised from the carcass. Excreta, carcass, skin and protective patch were assayed for ^{14}C . Penetration occurring over the 10 cm² of skin was quantified by addition of the ^{14}C levels in excreta and carcass.

2.4.2.1. Effect of occlusion and rinsing on penetration

Twelve rats were divided into 4 groups of 3 and treated with 1% (w/v) [¹⁴C] Octopirox in 50% shampoo. Two groups were fitted with protective patches (one occlusive and the other non-occlusive) without rinsing the skin. The remaining two groups of rats were rinsed with water after 10 minutes, the rinsings collected and the skin dried. One group was fitted with occlusive patches and the other with non-occlusive patches. The animals were returned to their metabolism cages and treated as above (EXP 1). The "10 minute application and rinsing regime" was repeated with two groups of 3 rats on a separate occasion (EXP 2).

2.4.2.2. Effect of duration of contact on penetration

Twelve rats were divided into 4 groups of 3. Each group was treated with 50% shampoo containing 1% (w/v) [¹⁴C] Octopirox in contact with the skin for a total of 2.5, 5, 10 or 20 minutes respectively before rinsing the skin. The skin was dried and the treated area of skin protected with an occlusive patch. The animals were then returned to their metabolism cages and treated as above.

2.4.2.3. Effect of concentration on penetration

Twelve rats were divided into 4 groups of 3. Groups were treated with 0.1, 0.25, 0.5 and 1.0% (w/v) [¹⁴C] Octopirox in 50% shampoo for 5 minutes, rinsed, dried and fitted with occlusive protective patches. The animals were returned to their metabolism cages and treated as above.

An extra group of 3 rats at each concentration of [¹⁴C] Octopirox was treated and sacrificed after rinsing to assay zero time skin deposition.

2.4.2.4. Effect of hair on penetration

In the experiment in which the effect of hair on penetration was studied a group of 3 animals was clipped 24 hours before treatment in such a way as to leave 10 cm² of hair on the back. Another group of 3 clipped rats was included for comparison. The animals were treated with a 50% shampoo solution containing 1% (w/v) [¹⁴C] Octopirox for 5 minutes before rinsing. The treated area was dried, protected with an occlusive patch and the rats returned to their metabolism cages and treated as above. This experiment was repeated using non occlusive patches.

2.4.2.5. Blood and tissue levels of Octopirox

Twelve rats were divided into two groups of six. One group was intubated orally with 0.126% [¹⁴C] Octopirox in 50% (w/v) aqueous polyethylene glycol. The second group was treated topically with 1% Octopirox on 10 cm² of clipped skin. Duration of contact was for 5 minutes after which the treated skin was dried without rinsing and covered with protective occlusive patches.

At 1h, 2h, 4h, 6h, 24h and 48h after treatment, one rat from each group was placed under cyclopropane anaesthesia and heart blood was withdrawn, well mixed in an anticoagulant vial for assay of ^{14}C . The animals were then sacrificed by cervical dislocation and dissected to remove selected tissues for processing and assay of ^{14}C . This experiment was repeated.

A further two groups of 6 rats were treated as the second group (above), but the treated skin was rinsed and protected with a non-occlusive patch. Thereafter, treatment of the animals was as described above.

2.5. Analyses of radioactivity

Urinary excretions inclusive of cage washings in the turnover study were diluted to 25 ml. For topical studies the actual volume was noted and undiluted samples were used. 0.5 ml aliquots were added to 10 ml of Beckman Ready Solve EP scintillator and counted in duplicate or triplicate.

All rat rinsings and aliquots of test doses administered or topically applied were made to known volumes with distilled water and 0.5 ml were counted as above.

Faecal samples were freeze dried and ground with a pestle and mortar to a fine powder. Accurately weighed, duplicate pellets of about 300 mg were made with a pellet machine for each sample. These were oxidised in a Packard tri-carb oxidiser and the $^{14}\text{CO}_2$ was trapped in an organic phase for ^{14}C counting.

Tissues were burnt either wet if small, or freeze dried, pelleted as above, oxidised and counted for ^{14}C .

Blood was absorbed on to combustopads for oxidation.

Carcasses were digested with alkali, skin residues with soluene and counted as aqueous samples.

In the first skin penetration study (2.4.2.1.), patches were soaked in 50 ml distilled water containing traces of shampoo base. For subsequent topical studies, they were soaked in methanol. 0.5 ml aliquots were counted for ^{14}C .

All measurements of ^{14}C were made on a Packard Spectrometer Model 2450 using standard liquid scintillation counting techniques.

Normal calculations of standard deviations were performed and these were used in the standard Student 't' test to determine the significance of results.

2.6. Metabolism of [^{14}C] Octopirox

The individual 0-24 hr urinary and faecal outputs were pooled into seven separate groups of 3 male and 3 female rats from treatments by intubation, injection (s.c. and i.p.) and 3 female rats after and topical application from the experiment investigating the combinations of occlusion/rinsing (Table 2).

Aliquots of urine (10 μ l) and of dried faeces (300 mg) were taken to determine the total radioactivity in each pooled sample in the turnover study. For topically treated animals, 0.5 ml urine samples were used to estimate total radioactivity.

Aliquots (10 μ l) of pooled urine samples were spotted on to TLC plates (5 x 20 cm, .25 mm polyamide Woelm) along with 2 standard spots of [14 C] Octopirox in methanol, one of which was added to non-radioactive urine. The TLC plates were developed in methanol:ethanolamine (99:1) and the position of the radioactivity assessed in the Spark Chamber (Birchover Inst., Hitchin). In addition, two 20 x 20 cm TLC plates were spotted with 30 μ l of the six pooled samples along with blank urine containing added [14 C] Octopirox and developed in water:propanol (60:40). Bands of silica, 1 cm wide, were scraped from the plates and counted in 1 ml scintillator in a Prias counter (Packard).

The corresponding faecal samples were extracted successively with methanol, twice with chloroform and finally with methanol. The extracts were clarified either by centrifugation or filtration. Aliquots of the chloroform extracts were evaporated to dryness under nitrogen and the residues dissolved in methanol. Aliquots of these and of the methanol extracts were counted to determine the total extractable radioactivity. Samples of the individual methanol and chloroform extracts were spotted on to polyamide TLC plates, developed in methanol:ethanolamine (99:1) and viewed in the spark chamber.

Urine and faeces from rats treated topically, unrinsed and protected with occlusive patches were also examined for metabolites of [14 C] Octopirox.

3. RESULTS

3.1. Characterisation of [14 C] Octopirox

The specific activity of [14 C] Octopirox according to our estimation was $15.56 \pm 0.11 \mu\text{Ci} \times \text{mg}^{-1}$ which was about 10% lower than the value of $17.56 \mu\text{Ci} \times \text{mg}^{-1}$ quoted by Hoechst for the batch supplied. TLC of [14 C] Octopirox in two solvent systems, methanol-ethanolamine (99:1) and H_2O -propanol (60:40) gave single spots (Rf. 0.8-0.9 when applied as methanolic solution, and 0.71-0.79 when used as a marker in blank urine). The radiochemical purity of [14 C] Octopirox, as judged by scraping 1 cm bands of TLC plates developed in the above two solvent systems, eluting and counting, was greater than 90%. Hoechst examined the sample of [14 C] Octopirox by TLC in four different solvent systems and quoted a radiochemical purity $\geq 98\%$.

3.2. Turnover of [14 C] Octopirox

The recoveries of ^{14}C from male and female rats dosed with [14 C] Octopirox are detailed in Appendix 1. and summarised in Table 1.

The data show that for the various routes of administration, between 65 and 87% of the dose was excreted in the faeces, while the urine contained between 6 and 19% of the dose. Together, the urine and faeces accounted for at least 82% of the dose, most of which was recovered during the first 24 hours after administration.

There was no measurable ^{14}C in the expired air during the first 24 hours after administration. This assay, therefore, was omitted in subsequent skin penetration studies. The distribution of radioactivity was similar for both sexes. At 96 hours after administration, kidneys, bladder, spleen and blood had no detectable radioactivity (Appendix 1). Liver contained less than 0.05% and the carcass remains less than 1% of the dose. Recoveries of ^{14}C in the excreta and carcass at 48 hours after treatment were used therefore to quantify skin penetration of [^{14}C] Octopirox in later experiments.

TLC of the first 24 hours urines and extracts of faeces (which removed 80% of the ^{14}C in the faeces) showed that [^{14}C] Octopirox was excreted apparently unchanged. Neither the sex of the rat nor the route of administration affected the metabolism of [^{14}C] Octopirox. As regards urinary metabolites, the chromatographic patterns were essentially similar to those of [^{14}C] Octopirox added to blank rat urine. However, because of streaking on the plates, the presence or absence of other minor metabolites in the urine could not be ascertained.

3.3. Skin penetration of [^{14}C] Octopirox

3.3.1. Effect of occlusion and rinsing

The recoveries of ^{14}C after topical application of 1% (w/v) [^{14}C] Octopirox in anionic shampoo base are detailed in Appendix 2.1. and summarised in Tables 2 and 2a.

In the absence of rinsing, a maximum skin penetration of $64.4 \mu\text{g}/\text{cm}^2$ was recorded with occlusion of the treated site, whereas non-occlusive protection reduced penetration to $38.2 \mu\text{g}/\text{cm}^2$. Total recoveries were reduced by incomplete extraction with water (27%) of ^{14}C from the protective patches (Appendix 2.2.). In all subsequent studies, patch bound ^{14}C recoveries were much improved by using extraction with methanol, without affecting the validity of the skin penetration data. The recoveries of ^{14}C in the excreta of days 1 and 2 were about the same in the occluded and non-occluded groups and accounted for some 30% of the applied [^{14}C] Octopirox in the occluded group and about 15% in the non-occluded group.

After rinsing, about 90% of the applied [^{14}C] Octopirox was recovered in the rinse water. There was good experimental agreement of skin penetration for the occluded group, 3.5 and $3.3 \mu\text{g}/\text{cm}^2$ and for the non-occluded group 2.2 and $1.8 \mu\text{g}/\text{cm}^2$ (Tables 2 and 2a). Recoveries of ^{14}C in the excreta were greater on day 1 than on day 2, especially in the faeces and accounted for 1.6% of the applied [^{14}C] Octopirox in the occluded group and 0.9% in the non-occluded group.

In these and subsequent skin penetration studies, as in the turnover study, more ^{14}C was recovered in the faeces than in the urine.

Thus, occlusion of the skin, with or without rinsing, increased skin penetration of [^{14}C] Octopirox by a factor of about 1.6. Rinsing of the skin with or without occlusion reduced skin penetration of [^{14}C] Octopirox by a factor of about 20.

3.3.2. Effect of duration of contact

The recoveries of ^{14}C after topical application of 1% [^{14}C] Octopirox in shampoo base at four different durations of contact are detailed in Appendix 3 and summarised in Table 3.

Over 85% of the applied amounts was recovered in the rinsings. The total ^{14}C recoveries in the rinsings, excreta, carcass, skin and patch were at least 90%. The total 48 hours ^{14}C recoveries in the excreta varied between 1 and 2% of the applied amount for different durations of contact. Recoveries of ^{14}C in the excreta during the first 24 hours were three to five times greater compared with those in the second 24 hours. Furthermore, faecal recoveries of ^{14}C were greater than the urinary recoveries.

The skin penetration values ranged from $2.4 \mu\text{g}/\text{cm}^2$ for 2.5 minutes' duration of contact to $4.4 \mu\text{g}/\text{cm}^2$ for 10 minutes' duration of contact. The penetration values at 2.5, 5 and 10 minutes' duration of contact are significantly ($P = 0.05$) different from each other (Table 3). However, the skin penetration of $4.2 \mu\text{g}/\text{cm}^2$ observed after 20 minutes' duration of contact is not significantly ($P = 0.05$) different from that observed after 10 minutes' duration of contact. Thus, there is a dependence of skin penetration of [^{14}C] Octopirox on duration of contact of up to 10 minutes.

3.3.3. Effect of concentration

The recoveries of ^{14}C after topical application of [^{14}C] Octopirox at four different concentrations in shampoo base are detailed in Appendix 4.1. The ^{14}C recoveries from rats, sacrificed to assay zero time deposition of Octopirox at these concentrations are listed in Appendix 4.2. These results are summarised in Table 4.

At all concentrations, over 95% of the applied amounts were recovered in the rinsings (Table 4). The total recoveries of ^{14}C in the rinsings, excreta, patch, skin residue and carcass ranged from 98 to 106% of the applied amounts. Recoveries of ^{14}C in the excreta during the first 24 hours were about 3 to 5 times greater than the recoveries during the second 24 hours (Appendix 4.1). The 48 hours recoveries of ^{14}C in the excreta varied from 1.2 to 1.6% of the amounts applied. Faecal recoveries of ^{14}C were, as in previous studies, greater than urinary recoveries.

The skin penetration was proportional to concentration between 0.1 and 1% Octopirox (Table 4) ranging from $0.31 \mu\text{g}/\text{cm}^2$ at 0.1% Octopirox to $3.6 \mu\text{g}/\text{cm}^2$ at 1% Octopirox, an increase of about tenfold.

The skin deposition of Octopirox, both at zero (Appendix 4.2) time and at 48 hours was also proportional to concentration. The values for deposition of Octopirox immediately after rinsing were respectively $0.8 \mu\text{g}/\text{cm}^2$ at 0.1% Octopirox and $7.6 \mu\text{g}/\text{cm}^2$ at 1% Octopirox, or about 4% of the applied amount.

3.3.4. Effect of hair

The recoveries of ^{14}C after topical application of [^{14}C] Octopirox to hairy and clipped skin are detailed in Appendix 5.1 and 5.2, and summarised in Tables 5 and 5a.

For the groups of rats fitted with occlusive (Table 5) or non-occlusive (Table 5a) patches, the rinse water contained 93-96% of the applied [^{14}C] Octopirox. The total recovery from all groups of animals was at least 96%. As expected from previous studies (Tables 2 and 2a) the recovery of the applied ^{14}C in the excreta was greater in the groups of animals fitted with occlusive patches (1.3%) compared to those fitted with non-occlusive patches (0.6%). Regardless of the nature of the protective patch, however, the presence of hair did not significantly ($P = 0.05$) affect the penetration of [^{14}C] Octopirox.

Despite the presence of hair, small amounts of Octopirox were deposited in skin. Immediately after rinsing (Appendix 5.2), the hair covering the treated area contained about 2.4 μg of Octopirox while the skin, after removal of the hair by clipping contained $4.4 \mu\text{g}/\text{cm}^2$. Clipped skin, immediately after application contained $7.5 \mu\text{g}/\text{cm}^2$, approximately twice as much as the skin from the unclipped rats.

All the data on skin penetration are summarised in Table 7, from which it can be seen that, by and large, there is good agreement between the various experiments for the effects of duration of contact, applied concentration, rinsing or not and occlusion.

3.4. Blood and tissue levels of [^{14}C] Octopirox

The distribution of [^{14}C] Octopirox in selected tissues and blood at times up to 48 hours are detailed for oral administration in Appendices 6.1 and 6.2 and for topical application in Appendices 7.1, 7.2 and 8.

The blood levels after oral intubation or topical application are summarised in Table 6.

The maximum blood level after oral intubation of 4.8 mg/kg body weight is $0.137 \mu\text{g}/\text{ml}$ at 2 hours after intubation and falls steadily thereafter to $0.007 \mu\text{g}/\text{ml}$ at 48 hours.

After topical application of 15.4 mg/kg body weight, without rinsing of the skin and protection of the skin with an occlusive patch, blood levels were $0.21 \mu\text{g}/\text{ml}$ to $0.32 \mu\text{g}/\text{ml}$ between 1 and 6 hours respectively, and fell rapidly at 24 and 48 hours. When the treated

skin was rinsed and protected with a non-occlusive patch the blood levels were much reduced by at least an order of magnitude (0.018 to 0.007 µg/ml at 1 and 6 hours respectively) compared to the no rinse, occlusion treatment known to enhance penetration (Table 6).

After oral administration of 4.8 mg/kg body weight, most of the radioactivity remained in the intestinal tract with maximum amounts in the small and large intestines at 2 and 6 hours respectively. The liver contained small amounts, the maximum of which was about 3 µg at 6 hours after intubation, whereas the kidney contained only about 10% of the liver. The other tissues examined, brain, eyes, lungs, heart, spleen, ovaries, all contained very low levels of radioactivity equivalent to a few nanograms of Octopirox, or less than 0.02% of the dose.

After topical application of 15.4 mg/kg body weight, without rinsing and with an occlusive protective patch covering the skin, tissue levels were similar to those after oral intubation. Most of the radioactivity was again associated with the gastrointestinal tract, the liver and kidney contained smaller amounts and the brain, eyes, lungs, heart, spleen, adrenals and ovaries all contained very low levels. The low ¹⁴C recoveries from rats sacrificed at 48 hours after treatment are very probably due to low faecal output for some unknown reasons (Appendices 7.1. and 7.2.)

When the skin was rinsed at 5 minutes after application and protected with a non-occlusive patch, the levels of radioactivity in the intestinal tract and liver and kidney were 10% or less than those from the unrinsed animals with occlusive patches. Other tissues were not analysed.

3.5. Metabolism of [¹⁴C] Octopirox after percutaneous absorption

Extraction of urine and faeces recovered 84% and 80% respectively of the total radioactivity of the individual or combined samples.

Examination of the extracted radioactivity on TLC revealed unchanged Octopirox as the dominant, recognisable component although the presence of traces of minor metabolites cannot be excluded. Furthermore, the nature of the small amount of unextracted radioactivity from urine and faeces is unresolved.

4. DISCUSSION

In the turnover experiments, both sexes of rats given [¹⁴C] Octopirox by three different routes of administration excreted large amounts (65 to 85%) in the faeces and much smaller amounts (6 to 19%) in the urine during 4 days. Female rats tended to excrete more in the urine than males. No radioactivity was exhaled during the first 24 hours, during which time most of the excreted radioactivity was recovered. In the second to fourth days, excretion of radioactivity was much reduced, usually less than 5% of the total excretion during 4 days. At the end of 4 days, the residual radioactivity in the carcass was always less than 1% of the dose. Thus, [¹⁴C] Octopirox was rapidly and virtually completely removed from the body. The nature of the radioactivity was confirmed by comparative chromatography to be largely unchanged Octopirox from the intubation and injection experiments and also after application to the skin.

In the skin penetration experiments, occlusion with or without rinsing of the skin, approximately doubled penetration of [^{14}C] Octopirox. The effect of rinsing, however, was much more dramatic in reducing penetration by a factor of twenty. The data show that Octopirox has a high potential for penetration, but that under conditions of use by the consumer of shampoo products which are rinsed away, the actual penetration is much reduced. This reduction is reflected in the demonstrably lower blood levels of radioactivity after skin application and rinsing.

Other factors, important in the use of shampoo products, were the effect on penetration of [^{14}C] Octopirox of the duration of contact and of the applied concentration. The time course study showed that penetration increased slowly with time up to a 10-minute contact, while penetration increased in proportion to the applied concentration. In addition, skin penetration was not reduced by the presence of hair, so that more confidence can be placed on the data generated from the more easily manipulated clipped animals. From these data one can extrapolate to the likely body burden of Octopirox for the consumer under a variety of conditions.

In calculating the human body burden, it has been proposed that scalp skin has a permeability close to that of rat skin (Black and Howes, 1975; Ammenheuser and Warren, 1979) and that hand skin is half as permeable as scalp skin (Maibach et al., 1971). The area available for penetration is 500 cm^2 for scalp plus 850 cm^2 for both hands (Black and Howes, 1975). If one considers a consumer using a shampoo containing 1% Octopirox, then for a 10-minute contact with rat skin before rinsing and fitting a non-occlusive patch, the observed penetration was $2.0\text{ }\mu\text{g}/\text{cm}^2$ in 48 hours (mean figure from Tables 2 and 2a). Thus, the consumer would absorb $1000\text{ }\mu\text{g}$ through the scalp and $850\text{ }\mu\text{g}$ through the hands, a total dose of $1850\text{ }\mu\text{g}$, which for a 55 kg woman is $34\text{ }\mu\text{g}/\text{kg}$ body weight. However, when the shampoo is applied to the wetted scalp, there is a tenfold dilution so that the expected dose is $3.4\text{ }\mu\text{g}/\text{kg}$ body weight, as penetration is proportional to concentration.

There are no published data on the subacute toxicity of Octopirox. However, Hoechst have conducted both a 90-day rat feeding study and a rabbit teratology study. The no effect level in the rat was $100\text{ mg}/\text{kg}/\text{day}$ and in the rabbit $53\text{ mg}/\text{kg}/\text{day}$ was the no-effect level (Gilpin, 1981). Thus, in relation to the no effect level from the teratology study, the calculated body burden of $3.4\text{ }\mu\text{g}/\text{kg}$ from one application of shampoo containing 1% Octopirox gives a safety factor of 18,500.^x This factor would be greater if the amount of Octopirox in the shampoo was reduced, or if the time to shampoo was reduced from our experimental figure of 10 minutes. Correspondingly, the safety factor would be less for higher concentrations of Octopirox in shampoo or if the scalp integrity was affected by damage or disease. However, the safety factor is very large, so that, on balance, the possibility of systemic toxicity resulting from the use of shampoo containing 1% Octopirox is remote.

^x 29,400 in relation to the no effect level from 90-day rat feeding study

5. REFERENCES

Ammenheuser, M. M. and Warren, H. E. (1979). Detection of mutagens in the urine of rats following topical application of hair dyes. *Mutation Research* 66, 241-245.

Black, J. G. and Howes, D. (1975). Percutaneous absorption of Triclosan from toilet preparations. *J. Soc. Cos. Chem.* 26, 205-215.

Gilpin, G. R. (1981). Octopirox - Antidanoruff agent from Hoechst. Internal memo.

Howes, D. (1975). The percutaneous absorption of some anionic surfactants. *J. Soc. Cos. Chem.* 26, 47-63.

Kellner and Eckert (1980). Pharmacokinetic studies of Octopirox-¹⁴C after dermal, oral and intravenous administration to rats. Hoechst Study Research Report No. 01-L42-0325-80.

Maibach, H. I., Feldman, R. J., Milby, T. H. and Serat, W. F. (1971). Regional variation in percutaneous penetration in man: *Pesticides Arch. Environ. Health* 23, 208-211.

TABLE 1 Turnover of Octopirox in the rat

GROUP	NO. OF RATS	SEX	ROUTE	^{14}C	URINE	FAECES	SPLEEN	KIDNEYS	BLADDER	LIVER	CARCASS	TOTAL RECOVERY
I	2	M	ORAL	B	6.27±0.68	85.49±2.71	0.01	0.01	0.001	0.010	0.11 ±0.009	91.80±3.53
II	"	"	IP	"	9.72±8.03	84.35±8.94	"	"	"	0.027±0.030	0.25 ±0.02	94.34±0.90
III	"	"	SC	"	12.88±3.29	73.74±1.70	"	"	"	0.015±0.012	0.98 ±0.17	87.63±1.40
IV	"	F	ORAL	"	8.61±0.62	86.73±4.57	"	"	"	0.010	0.075±0.009	95.42±3.93
V	"	"	IP	"	13.51±0.68	68.67±0.75	"	"	"	0.045±0.005	0.29 ±0.11	82.60±0.06
VI	"	"	SC	"	18.78±2.22	65.23±1.41	"	"	"	0.015± 0.010	0.77 ±0.62	84.80±1.43

Results are expressed as percentage + S.D. of dose.

M = Male; F = Female; IP = Intraperitoneal; SC = Subcutaneous; B = Background radioactivity (50 dpm).

Each rat received 0.5 ml of 0.112% (w/v) [^{14}C] Octopirox (20.079×10^6 dpm) in (50% v/v) polyethylene glycol 200 by the stated route.

TABLE 2 Effect of occlusion and rinsing of skin on penetration of Octopirox

TYPE OF PATCH	RINSINGS	URINE	FAECES	CARCASS	SKIN RESIDUE	PATCH	TOTAL RECOVERY	PENETRATION $\mu\text{g}/\text{cm}^2$
Occlusive	None	140 \pm 30	472 \pm 33.2	39 \pm 6.6	476 \pm 80	78 \pm 9	1205 \pm 152	65.1 \pm 6.9
Non-occlusive	None	72 \pm 10	230 \pm 49	79.5 \pm 71.0	649 \pm 68.5	226 \pm 20	1258 \pm 83	38.2 \pm 4.8
Occlusive	1820 \pm 78	9.4 \pm 1.1	23.4 \pm 4.2	1.3 \pm 0.6	25 \pm 12.6	2.8 \pm 1.4	1882 \pm 72	3.5 \pm 0.6
Non-occlusive	1818 \pm 61.5	4.2 \pm 0.4	14.5 \pm 2.8	2.8 \pm 0.5	19.2 \pm 0.4	3.8 \pm 0.6	1863 \pm 60	2.2 \pm 0.2

Results are expressed as $\mu\text{g} \pm$ S.D. of Octopirox. (See Appendix 2.1. EXP 1)

Groups of 3 female rats were treated with 0.2 ml of 1% (w/v) [^{14}C] Octopirox (2003 μg , 26.688×10^6 dpm) over 10 cm^2 skin and rinsed or not as appropriate after 10 minutes. The skin was dried and protected with occlusive or non-occlusive patches.

Penetration was calculated from recoveries of ^{14}C in excreta and carcass at 48 hours after treatment.

TABLE 2a Effect of occlusion and rinsing of skin on penetration of Octopirox

TYPE OF PATCH	RINSINGS	URINE	FAECES	CARCASS	SKIN RESIDUE	PATCH	TOTAL RECOVERY	PENETRATION $\mu\text{g}/\text{cm}^2$
Occlusive	1918 + 36	8.6 \pm 1.2	24.6 \pm 4.6	B	14.4 \pm 4	27.6 \pm 3.6	1992 \pm 43.4	3.3 \pm 0.5
Non-occlusive	1891 \pm 35	6.0 \pm 3.6	9.6 \pm 0.4	2.2 \pm 3.8	18.6 \pm 2.2	45.6 \pm 7.4	1970 \pm 24.8	1.8 \pm 0.3

Results are expressed as $\mu\text{g} \pm$ S.D. of Octopirox. B = Background radioactivity - 50 dpm. (See Appendix 2.1. EXP 2)

Groups of 3 female rats were treated with 0.2 ml of 1% (w/v) [^{14}C] Octopirox (2000 μg , 24.717×10^6 dpm) over 10 cm^2 skin and rinsed after 10 minutes. The skin was dried and protected with occlusive or non-occlusive patches.

Penetration was calculated from recoveries of ^{14}C in excreta and carcass at 48 hours after treatment.

TABLE 3 Effect of duration of contact with skin on penetration of Octopirox

DURATION OF CONTACT MINUTES	RINSINGS	URINE	FAECES	CARCASS	SKIN RESIDUE	PATCH	TOTAL RECOVERY	PENETRATION $\mu\text{g}/\text{cm}^2$
2.5	1943 \pm 83	6.2 \pm 1	16.4 \pm 0.6	1.6 \pm 0.4	14.6 \pm 1.9	58 \pm 42	2039 \pm 42	2.4 \pm 0.1
5*	1878 \pm 19	7.6 \pm 1	23 \pm 3	2.4 \pm 1.6	13.6 \pm 5.1	76 \pm 30	1998 \pm 57	3.3 \pm 0.2
10	1703 \pm 142	11.4 \pm 2.2	30.2 \pm 0.6	2.9 \pm 1.4	17.1 \pm 3.2	49 \pm 20	1814 \pm 161	4.5 \pm 0.2
20	1817 \pm 123	10.5 \pm 3.5	29.3 \pm 7.9	2.5 \pm 1.4	20.3 \pm 2.8	30.5 \pm 5.4	1911 \pm 120	4.2 \pm 1.3

Results are expressed as $\mu\text{g} \pm$ S.D. of Octopirox.

Groups of 3 female rats were treated with 0.2 ml of 1% (w/v) [^{14}C] Octopirox (2000 μg , 26.674×10^6 dpm) over 10 cm^2 skin for the specified time before rinsing. The treated area was dried and protected with an occlusive patch.

Penetration was calculated from recoveries of ^{14}C in excreta and carcass at 48 hours after treatment.

*Calculations based on only 2 rats (see Appendix 3).

TABLE 4 Effect of concentration applied to skin on penetration of Octopirox

CONCENTRATION OF OCTOPIROX % w/v	AMOUNT APPLIED μ g	RINSINGS	URINE	FAECES	CARCASS	SKIN RESIDUE	PATCH	TOTAL RECOVERY	PENETRATION μ g/cm ²
0.1	200	192 \pm 0.4	0.6 \pm 0.1	1.9 \pm 0.3	0.7 \pm 0.1	2.0 \pm 0.3	4.0 \pm 0.8	201.6 \pm 4.4	0.31 \pm 0.03
"	"	189 \pm 20.4	NA	NA	NA	8.4 \pm 1.7	NA	197.4 \pm 19.4	
0.25	503	508 \pm 8	1.7 \pm 0.2	4.8 \pm 0.2	0.8 \pm 0.04	4 \pm 0.9	7.5 \pm 2.0	528 \pm 8.3	0.73 \pm 0.06
"	"	511 \pm 16.5	NA	NA	NA	22.4 \pm 4.7	NA	533 \pm 20.3	
0.51	1021	968 \pm 24	3.5 \pm 1.4	11.4 \pm 3.5	2.4 \pm 2.0	7.4 \pm 1.7	19.7 \pm 7	1012 \pm 32.8	1.73 \pm 0.40
"	"	999 \pm 31.6	NA	NA	NA	38.3 \pm 8.0	NA	1037 \pm 25.2	
1.012	2025	1905 \pm 39.3	7.6 \pm 0.5	26.5 \pm 4.2	1.8 \pm 0.4	16.0 \pm 5.0	24.5 \pm 4.4	1983 \pm 44.7	3.60 \pm 0.40
"	"	2027 \pm 42	NA	NA	NA	76.3 \pm 16.8	NA	2103 \pm 30.6	

Results are expressed as μ g \pm S.D. of Octopirox.

NA = Not applicable.

Groups of 3 female rats were treated with 0.2 ml of test solution over 10 cm² skin for 5 minutes before rinsing. The treated area was dried and protected with an occlusive patch.

Penetration was calculated from recoveries of ¹⁴C in excreta and carcass at 48 hours after treatment.

HAIR TYPE	RINSINGS	URINE	FAECES	CARCASS	SKIN RESIDUE	PATCH	TOTAL RECOVERY	PENETRATION $\mu\text{g}/\text{cm}^2$
Hairy	1864 \pm 32	8.1 \pm 0.9	18.6 \pm 3.7	7.4 \pm 6.8	56 \pm 8.2	14 \pm 0.6	1969 \pm 27	3.4 \pm 0.8
Clipped	1865 \pm 56	7 \pm 1.8	18.7 \pm 4.8	2.4 \pm 1.8	17.4 \pm 6.3	21.3 \pm 8.6	1932 \pm 42	2.8 \pm 0.5

Results are expressed as $\mu\text{g} \pm \text{S.D.}$ of Octopirox.

Groups of 3 female rats were treated with 0.2 ml of 1% (w/v) [^{14}C] Octopirox (2000 μg , 27.055×10^6 dpm) over 10 cm^2 skin for 5 minutes before rinsing. The treated area was dried and protected with an occlusive patch.

Penetration was calculated from recoveries of ^{14}C in excreta and carcass at 48 hours after treatment.

TABLE 5a Effect of hair on penetration of Octopirox

SKIN TYPE	RINSING	URINE	FAECES	CARCASS	SKIN RESIDUE	HAIR	PATCH	TOTAL RECOVERY	SKIN PENETRATION $\mu\text{g}/\text{cm}^2$
Hairy	1893 \pm 89	3.9 \pm 1.2	8.8 \pm 2.6	2.4 \pm 1.3	9.0 \pm 2.2	25.7 \pm 4.3	16.8 \pm 5.2	1960 \pm 91.4	1.5 \pm 0.5
	1917 \pm 34				44.6 \pm 6.6	24.1 \pm 2.7		1986 \pm 28	
Clipped	1854 \pm 20	4.0 \pm 0.8	9.8 \pm 1.8	1.4 \pm 0.6	12.2 \pm 1.8	NA	35.2 \pm 1.6	1917 \pm 15	1.5 \pm 0.2
	1948 \pm 31				75 \pm 11.6			2023 \pm 31	

Results are expressed as $\mu\text{g} \pm \text{S.D.}$ of Octopirox.

NA - Not applicable.

Groups of 3 female rats were treated with 0.2 ml of 1% (w/v) [^{14}C] Octopirox (2000 μg , 25.151×10^6 dpm) over 10 cm^2 skin for 5 minutes before rinsing. The treated area was dried and protected with a non-occlusive patch.

Penetration was calculated from recoveries of ^{14}C in excreta and carcass at 48 hours after treatment.

TABLE 6 Blood levels of Octopirox in rats

Treatment	μg equivalents/ml at time (hr)					
	1	2	4	6	24	48
Oral 4.8 mg/kg B.W.	0.110	0.137	0.076	0.070	0.008	0.007
Topical 15.4 mg/kg B.W. (no rinsing, occlusion)	0.200	0.295	0.265	0.320	0.049	0.029
Topical 15.4 mg/kg B.W. (rinsed, non occlusion)	0.018	0.011	0.009	0.007	0.008	0.004

Results are the mean of 2 rats.

B.W. - Body weight.

In oral treatment, [^{14}C] Octopirox was given in 0.5 ml of 50% aqueous polyethylene glycol.

In topical treatments, [^{14}C] Octopirox at 1% was applied in 50% aqueous shampoo (0.2 ml) to 10 cm^2 clipped dorsal skin.

TABLE 7 Summary of effects of treatment on skin penetration of Octopirox

APPLIED CONCENTRATION (% w/v)	SKIN TREATMENT	DURATION OF CONTACT (min)	TYPE OF PATCH	SKIN PENETRATION ($\mu\text{g}/\text{cm}^2$)
1.0	No rinsing, clipped	48 hr.	Occlusive	65.1 + 6.0
			Non-occlusive	38.2 + 4.8
1.0	Rinsing, clipped	10 min	Occlusive	3.5 + 0.5
			Non-occlusive	2.2 + 0.2
1.0	Rinsing, clipped	10 min	Occlusive	3.3 + 0.6
			Non-occlusive	1.8 + 0.3
1.0	Rinsing, clipped	2.5 min	Occlusive	2.4 + 0.1
		5 min		3.3 + 0.2
		10 min		4.4 + 0.2
		20 min		4.2 + 1.3
0.1 0.25 0.51 1.01	Rinsing, clipped	5 min	Occlusive	0.3 + 0.03
				0.7 + 0.06
				1.7 + 0.4
				3.6 + 0.4
1.0	Rinsing, hairy clipped	5 min	Occlusive	3.4 + 0.8
				2.8 + 0.5
1.0	Rinsing, hairy clipped	5 min	Non-occlusive	1.5 + 0.5 1.5 + 0.2

Appendix 1 Turnover of [^{14}C] Octopirox in the rat

RAT	SEX	ROUTE	$^{14}\text{CO}_2^*$	URINE					FAECES				SPLEEN	KIDNEYS	LIVER	BLADDER	BLOOD x ml ⁻¹	CARCASS
				6h	6-24h	Day 2	Day 3	Day 4	Day 1	Day 2	Day 3	Day 4						
1	M	ORAL	B	0.328	0.605	0.181	0.035	0.012	16.356	0.382	0.035	0.008	B	0.001	0.001	B	2B	0.021
2	"	"	"	0.214	0.881	0.193	0.049	0.018	16.557	0.875	0.058	0.063	"	"	0.002	"	"	0.021
3	"	IP	"	0.143	0.494	0.117	0.037	0.020	17.554	0.493	0.136	0.023	"	"	0.001	2B	"	0.053
4	"	"	"	1.670	1.112	0.235	0.055	0.019	15.341	0.270	0.038	0.018	0.001	"	0.010	2B	"	0.047
5	"	SC	"	0.528	0.963	0.433	0.133	0.062	13.981	0.826	0.166	0.076	2B	"	0.005	2B	"	0.223
6	"	"	"	0.351	1.692	0.707	0.225	0.078	12.541	1.539	0.335	0.151	2B	"	0.001	2B	"	0.173
7	F	ORAL	"	0.456	1.003	0.152	0.019	0.010	17.872	0.176	0.011	0.005	B	"	0.001	"	"	0.013
8	"	"	"	0.533	1.122	0.134	0.020	0.008	15.147	1.594	0.019	0.007	"	"	"	"	"	0.017
9	"	IP	"	0.189	2.147	0.402	0.055	0.017	13.199	0.447	0.030	0.007	0.001	"	0.008	"	"	0.075
10	"	"	"	0.193	2.165	0.197	0.039	0.024	13.608	0.216	0.041	0.030	2B	"	0.01	"	"	0.042
11	"	SC	"	0.123	2.634	0.552	0.103	0.043	12.683	0.500	0.097	0.019	"	"	0.003	"	"	0.068
12	"	"	"	0.603	2.638	0.657	0.146	0.044	11.400	1.182	0.188	0.127	"	"	0.002	"	"	0.244

Results are expressed as dpm x 10⁻⁶ of ^{14}C . * $^{14}\text{CO}_2$ was not monitored after first 24 hours.

M = Male; F = Female; IP = Intraperitoneal; SC = Subcutaneous; B = Background (50 dpm).

Each rat received 0.5 ml of 0.112% (w/v) [^{14}C] Octopirox (20.079 x 10⁶ dpm) in 50% (v/v) polyethylene glycol 200 by the stated route.

Appendix 2.1 Effect of occlusion and rinsing of skin on penetration of [¹⁴C] Octopirox

RAT EXP 1	TYPE OF PATCH	RINSINGS	URINE			FAECES			CARCASS	SKIN RESIDUE	PATCH*	TOTAL RECOVERY
			Day 1	Day 2	Sum	Day 1	Day 2	Sum				
1	Occlusive	None	0.978	1.076	2.054	2.871	3.446	5.317	0.566	7.137	1.009	17.083
2			1.137	0.998	2.135	3.597	3.922	6.438	0.582	6.757	1.177	17.370
3			0.955	0.441	1.400	3.590	2.245	5.835	0.419	5.131	0.937	13.722
4	Non- Occlusive	None	0.400	0.536	0.936	1.438	1.514	2.952	0.462	9.105	2.790	16.246
5			0.311	0.534	0.845	1.315	1.165	2.480	2.148	9.243	3.311	18.027
6			0.418	0.687	1.105	1.628	2.151	3.779	0.565	7.598	2.956	16.003
7	Occlusive	24.250	0.101	0.029	0.130	0.308	0.057	0.365	0.036	0.520	0.060	25.361
8		23.216	0.109	0.027	0.136	0.292	0.027	0.319	0.029	0.280	0.027	24.007
9		25.289	0.082	0.027	0.109	0.204	0.049	0.251	0.013	0.195	0.027	25.886
10	Non- Occlusive	23.505	0.040	0.022	0.062	0.143	0.041	0.184	0.039	0.250	0.061	24.101
11		24.062	0.041	0.011	0.052	0.207	0.029	0.236	0.021	0.261	0.045	24.686
12		25.119	0.038	0.015	0.053	0.122	0.039	0.161	0.045	0.262	0.047	25.688
EXP 2	Occlusive	24.036	0.088	0.025	0.113	0.320	0.045	0.365	B	0.218	0.375	25.107
2		23.874	0.071	0.018	0.089	0.216	0.037	0.253	*	0.120	0.362	24.698
3		23.205	0.113	0.018	0.131	0.253	0.037	0.290	*	0.168	0.290	24.068
4	Non- Occlusive	23.529	0.028	0.009	0.037	0.092	0.029	0.121	0.085	0.229	0.474	24.475
5		22.884	0.065	0.024	0.089	0.072	0.042	0.114	B	0.258	0.658	24.003
6		23.702	0.050	0.011	0.061	0.101	0.019	0.120	*	0.203	0.560	24.646

Results are expressed as dpm x 10⁻⁶ of ¹⁴C.

B = Background (50 dpm).

Each female rat was treated with 0.2 ml of 1% (w/v) [¹⁴C] Octopirox over 10 cm² skin. In the first experiment, rats 1-12 received 2003 µg, 26.688 x 10⁶ dpm, and in the second experiment rats 1-6 received 2000 µg, 24.717 x 10⁶ dpm of [¹⁴C] Octopirox.

The skin was rinsed after 10 minutes, dried and protected with occlusive or non-occlusive patches.

The rats were left in individual metabolism cages for 48 hours. Urine and faeces were separated and collected every 24 hours and the animals killed at 48 hours after treatment.

*Patches of rats 1-12, Expt. 1, extracted with water, other patches extracted with methanol (see also Appendix 2.2).

Appendix 2.2 Extraction of [¹⁴C] Octopirox from patches

GROUP	PATCH NO.	SOLVENT FOR EXTRACTION	AMOUNT RECOVERED dpm x 10 ⁻⁶	PER CENT RECOVERY OF ¹⁴ C	GROUP MEAN PER CENT RECOVERY
I	1	Water	8.576	32.13	26.76 ± 4.65
	2		6.410	24.02	
	3		6.438	24.12	
II	4	Methanol	22.071	82.70	84.11 ± 1.60
	5		22.363	83.80	
	6		22.910	85.84	

0.2 ml of 1% (w/v) Octopirox (26.688×10^6 dpm) was applied to 10 cm^2 of lint moistened with 0.4 ml of water. The patches were placed under a cardboard cover for 3 days before extraction with 50 ml water containing a trace of shampoo base or 50 ml methanol. Aliquots (0.5 ml) were used to measure radioactivity. (See Appendix 2.1. EXP 1)

Appendix 3 Effect of duration of contact with skin on penetration of [¹⁴C] Octopirox

RAT	DURATION OF CONTACT Minutes	RINSINGS	URINE			FAECES			CARCASS	SKIN RESIDUE	PATCH	TOTAL RECOVERY
			Day 1	Day 2	Sum	Day 1	Day 2	Sum				
1	2.5	26.472	0.073	0.018	0.091	0.193	0.027	0.220	0.017	0.160	0.668	27.629
2		24.639	0.074	0.018	0.092	0.193	0.033	0.226	0.018	0.205	1.380	26.560
3		26.629	0.050	0.015	0.065	0.178	0.030	0.208	0.025	0.201	0.271	27.399
4*	5	12.496	0.106	0.021	0.127	0.284	0.030	0.314	0.023	0.236	0.328	13.524
5		25.196	0.087	0.025	0.112	0.298	0.040	0.338	0.019	0.230	1.295	27.190
6		24.833	0.068	0.025	0.093	0.228	0.050	0.278	0.048	0.133	0.729	26.114
7	10	24.322	0.083	0.040	0.123	0.269	0.142	0.411	0.060	0.181	0.905	26.002
8		23.189	0.149	0.032	0.181	0.349	0.048	0.397	0.037	0.240	0.686	24.745
9		20.615	0.119	0.033	0.152	0.309	0.090	0.399	0.021	0.264	0.376	21.827
10	20	22.554	0.106	0.028	0.134	0.343	0.057	0.400	0.025	0.286	0.337	23.736
11		25.829	0.078	0.020	0.098	0.241	0.041	0.282	0.020	0.228	0.405	26.863
12		24.331	0.150	0.041	0.191	0.417	0.074	0.491	0.055	0.299	0.481	25.848

Results are expressed as dpm x 10⁻⁶ of ¹⁴C.

Each female rat was treated with 0.2 ml of 1% (w/v) [¹⁴C] Octopirox (2000 µg, 26.674 x 10⁶ dpm) over 10 cm² for the specified time before rinsing with distilled water. The skin was dried and covered with a protective occlusive patch for 48 hours.

*Amount applied to rat no. 4 was, in error, about half the stipulated amount applied to the others and is therefore excluded from the group calculation.

Appendix 4.1 Effect of concentration applied to skin on penetration of [¹⁴C] Octopirox

RAT	CONCENTRATION OF OCTOPIROX % w/v	AMOUNT APPLIED dpm x 10 ⁻⁶	RINSINGS	URINE			FAECES			CARCASS	SKIN RESIDUE	PATCH	TOTAL RECOVERY
				Day 1	Day 2	Sum	Day 1	Day 2	Sum				
1	0.1	7.478	7.179	0.020	0.007	0.027	0.066	0.014	0.080	0.018	0.069	0.175	7.548
2			7.408	0.013	0.004	0.017	0.048	0.011	0.059	0.028	0.070	0.118	7.700
3			7.006	0.017	0.006	0.023	0.052	0.022	0.074	0.028	0.086	0.151	7.368
4	0.25	18.571	18.459	0.049	0.014	0.063	0.166	0.021	0.187	0.016	0.184	0.267	19.176
5			19.052	0.047	0.008	0.055	0.152	0.021	0.173	0.023	0.124	0.361	19.789
6			18.808	0.058	0.015	0.073	0.135	0.038	0.173	0.047	0.140	0.217	19.458
7	0.51	21.173	20.236	0.084	0.020	0.104	0.255	0.063	0.318	0.023	0.193	0.558	21.432
8			19.508	0.043	0.013	0.056	0.186	0.024	0.210	0.027	0.139	0.261	20.202
9			20.456	0.043	0.011	0.055	0.134	0.050	0.184	0.099	0.125	0.398	21.317
10	1.0	27.552	26.540	0.090	0.017	0.107	0.271	0.044	0.315	0.025	0.245	0.365	27.597
11			25.761	0.090	0.017	0.107	0.375	0.050	0.425	0.018	0.269	0.375	26.955
12			25.512	0.074	0.023	0.096	0.293	0.049	0.342	0.028	0.140	0.263	26.381

Results are expressed as dpm x 10⁻⁶ of ¹⁴C.

Each female rat was treated with 0.2 ml of test solution of [¹⁴C] Octopirox over 10 cm² skin for 5 minutes before rinsing with distilled water. The skin was dried and covered with a protective occlusive patch for 48 hours.

Appendix 4.2 Effect of concentration applied to skin on deposition of [¹⁴C]
OCTOPIROX

RAT	CONCENTRATION OF OCTOPIROX % w/v	AMOUNT APPLIED	RINSINGS	SKIN RESIDUES	TOTAL RECOVERY
13	0.1	7.478	7.567	0.242	7.809
14			7.442	0.351	7.793
15			6.188	0.355	6.543
16	0.25	18.571	18.642	0.647	19.249
17			18.400	0.851	19.251
18			19.560	0.990	20.550
19	0.51	21.173	20.075	0.988	21.063
20			20.730	0.693	21.423
21			21.387	0.706	22.093
22	1.012	27.552	27.357	0.959	28.316
23			28.230	0.863	29.093
24			27.151	1.297	28.449

Results are expressed as dpm x 10⁻⁶ of ¹⁴C.

Each female rat was treated with 0.2 ml of test solution of [¹⁴C] Octopirox over 10 cm² skin for 5 minutes before rinsing with distilled water. The animals were killed and the skin excised for assay of ¹⁴C.

RAT	SKIN TYPE	AMOUNT APPLIED	RINSINGS	URINE			FAECES			CARCASS	SKIN RESIDUE	HAIR	NON-OCCLUSIVE PATCH	TOTAL RECOVERY
				Day 1	Day 2	Sum	Day 1	Day 2	Sum					
1	Hairy	25.1514	23.531	0.052	0.015	0.067	0.130	0.019	0.149	0.046	0.144	0.386	0.262	24.586
2			22.847	0.035	0.007	0.042	0.085	0.018	0.103	0.014	0.092	0.294	0.139	23.532
3			25.033	0.031	0.007	0.038	0.071	0.011	0.082	0.030	0.107	0.289	0.236	25.815
4	Clipped	"	23.387	0.026	0.014	0.040	0.082	0.029	0.111	0.027	0.161	NA	0.438	24.164
5			23.034	0.039	0.015	0.054	0.128	0.021	0.149	0.018	0.174	"	0.466	23.895
6			23.529	0.041	0.018	0.059	0.092	0.018	0.110	0.012	0.129	"	0.425	24.264

RAT	SKIN TYPE	AMOUNT APPLIED	RINSINGS	URINE			FAECES			CARCASS	SKIN RESIDUE	OCCLUSIVE PATCH	TOTAL RECOVERY
				Day 1	Day 2	Sum	Day 1	Day 2	Sum				
7	Hairy	27.0555	24.788	0.094	0.029	0.123	0.270	0.037	0.307	0.069	0.845	0.183	26.312
8			25.656	0.078	0.031	0.109	0.186	0.050	0.236	0.204	0.638	0.188	27.031
9			25.224	0.076	0.022	0.098	0.175	0.036	0.211	0.026	0.814	0.200	26.573
10	Clipped	"	24.768	0.053	0.014	0.067	0.155	0.036	0.191	0.062	0.322	0.405	25.815
11			24.817	0.088	0.027	0.115	0.288	0.032	0.320	0.016	0.233	0.288	25.787
12			26.105	0.082	0.019	0.101	0.219	0.027	0.246	0.019	0.150	0.173	26.794

Figures are expressed as dpm x 10⁻⁶ of ¹⁴C.

NA - Not applicable.

Female rats were treated in two separate experiments with 0.2 ml of 1% (w/v) [¹⁴C] Octopirox over 10 cm² skin for 5 minutes before rinsing and drying the skin and fitting either an occlusive or a non-occlusive patch.

Appendix 5.2 Effect of hair on deposition of [^{14}C] Octopirox

RAT	SKIN TYPE	AMOUNT APPLIED	RINSINGS	SKIN RESIDUE	HAIR	TOTAL RECOVERY
13	Hairy	25.1514	24.072	0.635	0.272	24.979
14			23.709	0.575	0.336	24.620
15			24.558	0.473	0.301	25.332
16	Clipped	"	24.083	0.921	NA	25.004
17			24.554	1.100	"	25.654
18			24.872	0.812	"	25.684

Figures are expressed as dpm $\times 10^{-6}$ of ^{14}C .

NA - Not applicable.

Each female rat was treated with 0.2 ml of 1% (w/v) [^{14}C] Octopirox over 10 cm^2 skin for 5 minutes. The skin was rinsed with distilled water and dried. The animals were killed immediately and the skin and hair analysed separately for ^{14}C .

Appendix 6.1 Tissue levels of [¹⁴C] Octopirox after oral intubation

RAT TIME (h)	1 1	2 2	3 4	4 6	5 24	6 48
<u>TISSUE</u>						
Brain	0.006	0.057	0.012	0.025	<0.006	<0.006
Eyes	0.006	0.057	0.038	<0.006	<0.006	<0.006
Lungs	0.019	0.031	0.025	0.031	0.006	<0.006
Heart	0.012	0.019	0.012	0.019	0.006	<0.006
Liver	2.390	2.609	2.898	3.024	0.712	0.246
Spleen	0.012	0.036	0.063	0.019	0.006	<0.006
Stomach	402.6	325.100	216.720	47.787	0.62	0.033
Small intestines	127.3	233.730	103.320	103.950	6.00	1.071
Large intestines	0.252	37.800	189.00	323.570	3.15	1.26
Kidneys	0.441	0.240	0.227	0.380	0.057	0.006
Adrenals	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006
Ovaries	0.012	0.050	0.025	0.012	0.006	<0.006
Fat xg ⁻¹	0.018	0.063	0.038	0.032	0.038	0.019
Muscle xg ⁻¹	0.063	0.076	0.063	0.069	0.019	0.019
Blood xml ⁻¹	0.088	0.102	0.063	0.078	0.008	0.007
Carcass	3.3	4.030	2.583	4.725	1.72	0.63
Urine	0.006	8.442	14.238	21.990	112.80	151.30
Faeces	0.006	3.082	0.157	0.012	424.00	425.30
TOTAL	536.5	612.5	529.5	505.7	548.3	590.2

Results are ug equivalents of [¹⁴C] Octopirox from individual animals.

Female rats were intubated with 0.5 ml of 0.126% (w/v) [¹⁴C] Octopirox (630 µg, 23.831 x 10⁶ dpm) in 50% aqueous polyethylene glycol 200.

Appendix 6.2 Tissue levels of [^{14}C] Octopirox after oral intubation

RAT TIME (h)	1 1	2 2	3 4	4 6	5 24	6 48
<u>TISSUE</u>						
Brain	0.050	0.012	0.012	0.006	0.006	<0.006
Eyes	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006
Lungs	0.069	0.050	0.044	0.019	0.006	<0.006
Heart	0.025	0.019	0.019	0.031	0.006	<0.006
Liver	3.040	2.942	2.120	2.785	0.353	0.180
Spleen	0.012	0.019	0.012	0.006	<0.006	<0.006
Stomach	258.500	178.00	6.520	4.725	0.400	0.130
Small intestines	228.00	278.210	65.460	18.150	3.00	0.542
Large intestines	0.202	10.330	454.230	621.600	8.95	1.134
Kidneys	0.321	0.350	0.164	0.100	0.057	0.006
Adrenals	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006
Ovaries	0.025	0.019	0.031	0.151	<0.006	<0.006
Fat xg^{-1}	0.076	0.025	0.070	0.280	0.035	0.019
Muscle xg^{-1}	0.107	0.113	0.063	0.070	0.019	0.019
Blood xml^{-1}	0.133	0.173	0.090	0.063	0.008	0.007
Carcass	3.800	3.900	3.090	3.024	2.120	0.630
Urine	1.450	7.310	17.325	20.00	39.20	36.040
Faeces	0.082	0.53	0.006	0.044	462.00	479.30
TOTAL	495.90	482.00	549.3	503.4	514.7	518.0

Results are μg equivalents of [^{14}C] Octopirox from individual animals.

Female rats were intubated with 0.5 ml of 0.126% (w/v) [^{14}C] Octopirox (630 μg , 22.886×10^6 dpm) in 50% aqueous polyethylene glycol 200.

Appendix 7.1 Tissue levels of [¹⁴C] Octopirox after topical application

RAT TIME (h)	7 1	8 2	9 4	10 6	11 24	12 48
<u>TISSUE</u>						
Brain	1.18	0.060	0.060	0.080	0.020	<0.020
Eyes	0.20	0.180	<0.020	0.080	0.10	0.020
Lungs	0.080	0.080	0.060	0.08	0.040	0.020
Heart	0.040	0.040	0.040	0.040	0.020	<0.020
Liver	3.400	3.600	3.00	3.800	2.40	0.200
Spleen	0.020	0.020	0.020	<0.020	0.020	<0.020
Stomach	30.00	4.00	1.30	1.40	0.160	1.60
Small intestines	20.00	23.40	46.00	38.00	21.00	5.00
Large intestines	0.80	1.80	2.60	57.00	55.20	14.40
Kidneys	0.360	0.400	0.440	0.540	0.280	0.760
Adrenals	<0.020	<0.020	<0.020	<0.020	<0.020	<0.020
Ovaries	0.060	0.040	0.040	0.040	0.020	0.020
Fat xg ⁻¹	0.180	0.120	0.200	0.540	0.440	0.340
Muscle xg ⁻¹	0.180	0.380	0.120	0.140	0.140	0.040
Blood xml ⁻¹	0.150	0.180	0.150	0.180	0.060	0.020
Patch	470.00	343.40	358.00	348.20	420.20	563.00
Skin residue	2140.00	1440.00	1412.00	1246.00	678.00	454.00
Urine	<0.020	<0.020	6.60	7.40	122.00	89.40
Faeces	N.D.	N.D.	0.040	3.80	378.00	260.00*
TOTAL	2667.0	1818.0	1824.0	1707.3	1678.1	1389.0

Results are ug equivalents of [¹⁴C] Octopirox from individual animals.

N.D. - Not determined.

Female rats were treated with 0.2 ml of 1% (w/v) [¹⁴C] Octopirox (2000 µg, 29.131 x 10⁶ dpm) over 10 cm² clipped skin. The skin was not rinsed, but dried and protected with an occlusive patch.

Carcass estimations of ¹⁴C were not done.

* Incomplete faecal output.

Appendix 7.2 Tissue levels of [^{14}C] Octopirox after topical application

RAT TIME (h)	7 1	8 2	9 4	10 6	11 24	12 48
<u>TISSUE</u>						
Brain	0.480	0.080	0.140	0.080	<0.020	<0.020
Eyes	0.6	<0.020	<0.020	0.060	0.200	0.020
Lungs	0.300	0.180	0.260	0.180	0.020	<0.020
Heart	0.060	0.080	0.040	0.080	<0.020	<0.020
Liver	4.320	7.660	6.220	5.200	1.300	2.320
Spleen	0.020	0.040	0.040	0.080	<0.020	<0.020
Stomach	2.320	0.440	1.760	3.080	2.600	0.140
Small intestines	24.400	82.00	74.20	60.50	15.00	9.80
Large intestines	0.440	3.020	80.00	134.30	31.00	10.20
Kidneys	0.760	0.400	0.980	0.280	0.060	0.140
Adrenals	<0.020	0.020	0.020	<0.020	<0.020	<0.020
Ovaries	0.08	0.040	0.040	0.080	0.020	0.020
Fat xg^{-1}	0.240	0.060	0.300	0.500	0.560	0.140
Muscle xg^{-1}	1.980	0.300	0.400	0.600	0.160	0.060
Blood xm^{-1}	0.250	0.410	0.380	0.460	0.038	0.037
Patch	454.00	432.00	440.00	489.06	667.00	414.00
Skin residue	1357.00	1321.00	1280.00	1166.4	641.70	401.20
Urine	0.02	0.360	15.60	21.40	72.60	141.00
Faeces	0.400	0.020	5.00	15.60	337.00	419.20*
TOTAL	1847.7	1848.1	1905.4	1898.0	1769.3	1398.1

Results are ug equivalents of [^{14}C] Octopirox from individual animals.

Female rats were treated with 0.2 ml of 1% (w/v) [^{14}C] Octopirox (2000 μg , 29.131×10^6 dpm) over 10 cm^2 clipped skin. The skin was not rinsed, but dried and protected with an occlusive patch.

Carcass estimations of ^{14}C were not done.

* Incomplete faecal output.

Appendix B Tissue levels of [^{14}C] Octopirox after topical application

GROUP	I	II	III	IV	V	VI
Time of Sacrifice (h)	1	2	4	6	24	48
<u>TISSUE</u>						
Blood ml^{-1}	0.018 \pm 0.003	0.011 \pm 0.001	0.009 \pm 0.001	0.007 \pm 0.003	0.008 \pm 0.004	0.004 \pm 0.002
liver	0.27 \pm 0.05	0.12 \pm 0.05	0.12 \pm 0.05	0.08 \pm 0	0.03 \pm 0.001	\bar{B}
Kidneys	0.025 \pm 0.035	0.025 \pm 0.009	0.016 \pm 0.02	0.015 \pm 0.01	\bar{B}	0.011 \pm 0.001
Small Int.	2.23 \pm 0.61	3.20 \pm 0.94	1.76 \pm 0.60	1.29 \pm 0.06	0.27 \pm 0.05	0.08 \pm 0
Large Int.	\bar{B}	0.12 \pm 0.16	1.88 \pm 0.55	2.42 \pm 0	0.74 \pm 0.27	0.012 \pm 0
Urine	N.D.	N.D.	N.D.	N.D.	1.9 \pm 1.0	2.3 \pm 0.16
Faeces	"	"	"	"	7.3 \pm 0.94	10.2 \pm 1.2
Skin Residue 10 cm^2	45 \pm 1.2	40.6 \pm 14	42 \pm 5.4	28.6 \pm 5.4	20.7 \pm 1.4	13.6 \pm 0.28
Patch	16.4 \pm 0.6	53 \pm 43	36 \pm 14.6	31.3 \pm 7.2	37.1 \pm 5.1	38.3 \pm 8.2
Rinsings	1926 \pm 48	1935 \pm 37	1903 \pm 22	1888 \pm 10	1860 \pm 41	1927 \pm 18
Total	1990 \pm 49	2032 \pm 19	1985 \pm 1.2	1952 \pm 3	1928 \pm 37	1992 \pm 8

Results are μg equivalents of [^{14}C] Octopirox and are the mean of 2 female rats.

B = Background (50 dpm).

N.D. - Not determined.

Each rat was treated with 0.2 ml of 1% (w/v) [^{14}C] Octopirox (2000 μg , 25.579×10^6 dpm) over 10 cm^2 clipped skin for 5 minutes before rinsing with distilled water. The skin was dried and protected with a non-occlusive patch.