| **Section A6.2**<br>**Annex Point IIA VI.6.2** | **Percutaneous absorption (in vivo test)**<br>**In-vivo dermal absorption in rat dermis** | |
|---|---|---|
| | **1 REFERENCE** | **Official use only** |
| **1.1 Reference** | [redacted]; In vivo percutaneous absorption of an EC formulation of [ C] Cypermethrin in rats. [redacted].<br>Dates of experimental work: 26 January 2009 – 16 February 2009 | |
| **1.2 Data protection** | Yes | |
| 1.2.1 Data owner | Agriphar S.A. | |
| 1.2.2 Companies with letter of access | | |
| 1.2.3 Criteria for data protection | Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I | |
| | **2 GUIDELINES AND QUALITY ASSURANCE** | |
| **2.1 Guideline study** | Yes<br>OECD guideline for the testing of chemicals: Guideline no. 427; Skin Absorption: *in vivo* method (Paris, April 2004).<br>OECD Environmental Health and Safety Publications, Series on Testing and Assessment no. 28. Guidance document for the conduct of skin absorption studies, Paris, March 2004.<br>Opinion of the Scientific Committee on Plants on Commission Draft Guidance on Dermal Absorption SCP/Guide-DERM/002 (April 2002).<br>Guidance document on Dermal Absorption (SANCO 222/2000, rev 7, 19 March 2004). | |
| **2.2 GLP** | Yes | |
| **2.3 Deviations** | No | |
| | **3 MATERIALS AND METHODS** | |
| **3.1 Test material** | Cypermethrin<br>Molecular formula : $C_{22}H_{19}C_{l2}NO_3$<br>Molecular weight : 416.3<br>CAS registration number : 52315-07-08<br>Appearance : light brown liquid<br>Solubility in water (pH 7) : 3.97 µg.L-1<br>Partition coefficient (log Pow) : 5.5 at 20°C<br>Storage conditions : 2-10°C<br>Supplier : Agriphar, SA<br>Date of arrival : 24 November 2008<br>Expiry date : 1 June 2011<br><br>Cypermethrin 500 EC amd Cypermethrin 500EC placebo were also included in this asay. Details for these materials were: | |

| **Section A6.2**<br>**Annex Point IIA VI.6.2** | **Percutaneous absorption (in vivo test)**<br>**In-vivo dermal absorption in rat dermis** | |
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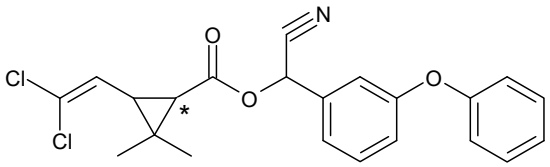
| | *Cypermethrin 500 EC*<br>Lot no. : 169606<br>Content : 499.7 $g.L^{-1}$<br>Appearance : light yellowish liquid<br>Storage conditions : 2-10°C<br>*Supplier : Agriphar, S.A.*<br><br>*Cypermethrin 500 EC placebo*<br>Other name : DIL 203/08 AW<br>Lot no. : BA0030/07<br>Content : 0 $g.L^{-1}$<br>Appearance : light yellowish liquid<br>Storage conditions : 2-10°C<br>Supplier : Agriphar, S.A. | |
| 3.1.1 Lot/Batch number | Lot no.: TJ230054801 | |
| 3.1.2 Specification | See 3.1 above | |
| 3.1.2.1 Description | The appearance of cypermethrin 40/60 TG was a light brown liquid (not a brown powder as described in the protocol). | |
| 3.1.2.2 Purity | Purity : 99% | |
| 3.1.2.3 Stability | Stable for duration of the study | |
| 3.1.2.4 Radiolabelling |  <br>Position of $^{14}C$ label<br>[$^{14}C$-cyclopropane] trans Cypermethrin<br>Lot : 07BLY094<br>Specific activity : 54.0 mCi/mmol<br>Amount received : 0.5 mCi<br>Molecular weight : 418.0 (at this specific activity)<br>Stated radiochemical purity : >98%<br>Storage conditions : ≤ -70°C<br>Date of arrival : 24 November 2008<br>Expiry date : 24 November 2013<br>Supplier : Blychem Ltd., UK | |
| **3.2 Test Animals** | | |
| 3.2.1 Species | Rat | |

| **Section A6.2**<br>**Annex Point IIA VI.6.2** | **Percutaneous absorption (in vivo test)**<br>**In-vivo dermal absorption in rat dermis** | |
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| 3.2.2 Strain | Wistar (Crl:(WI)WU BR) | |
| 3.2.3 Source | [redacted] | |
| 3.2.4 Sex | Males | |
| 3.2.5 Age/weight at study initiation | Young adults – 8 weeks old weighing approximately 250g.<br>The rats were acclimated to the laboratory environment for at least 5 days prior to treatment. Animals were kept in a room ventilated with 9-11 air changes per hour and maintained at a temperature of 22 +/- 3ºC. Relative humidity was maintained between 30-70%RH. Artificial light was on a 12h/12h light/dark daily cycle. | |
| 3.2.6 Number of animals per group | 24 males were used in the assay | |
| 3.2.7 Control animals | Yes | |
| **3.3 Administration/ Exposure** | Dermal | |
| 3.3.1 Preparation of test site | At least 20 hours prior to dosing each animal had an area of circa 20 $cm^2$ of skin clipped free of hair in the shoulder / dorsal region. Following clipping the area was wiped with acetone to remove fat and skin oils and then checked for abrasions. Only rats with intact skin were used for dose application.<br>After preparation, the animals were housed in metabolism cages. Prior to dosing, a non-absorbing phyton 'O'-ring with an inside area of approximately 10 $cm^2$ was glued to the clipped skin using cyanoacrylate adhesive. | |
| 3.3.2 Concentration of test substance | See Table IIIA 6.2-1 below.<br>[$^{14}$C]-Cypermethrin was dissolved in acetonitrile.<br>For dose preparation A, 7.5 MBq [$^{14}$C]-Cypermethrin, dissolved in acetonitrile, was transferred to a vial. Subsequently, the acetonitrile was evaporated to near-dryness under nitrogen gas, and 3.0 mL Cypermethrin 500 g/L EC (see above) was added.<br>For dose preparation B, 5.97 MBq [$^{14}$C]-Cypermethrin dissolved in acetonitrile, was transferred to a vial. Subsequently, the acetonitrile was evaporated to near-dryness under nitrogen gas, and 100 µL of Cypermethin 500 g/L EC placebo, 40 times diluted with demineralised water, was added. The formulation was sonicated for about 30 minutes. Then 9.90 mL demineralised water was added while vortexing the formulation.<br>The preparations were kept shielded from light in ambient temperature storage until required.<br>For Part A the nominal concentration was 500 g/L and for part B the nominal concentration was 25 mg/L. | |

| **Section A6.2**<br>**Annex Point IIA VI.6.2** | **Percutaneous absorption (in vivo test)**<br>**In-vivo dermal absorption in rat dermis** | |
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| 3.3.3 Specific activity of test substance | Prior to the start of the study, the radiochemical purity of [$^{14}$C]-Cypermethrin was verified with radio-HPLC analysis. Cypermethrin 40/60 TG was used to confirm the identity of the radiolabelled Cypermethrin. In addition, the radiochemical purity of [$^{14}$C]-Cypermethrin in the dose preparations was checked before each application.<br>The specific activity of the $^{14}$C- *trans*-cypermethrin supplied was 54.0 mCi/mmol. | |
| 3.3.4 Volume applied | Dermal application onto a dorsal area of about 10 cm$^2$, limited by an 'O'-ring under semi-occlusive conditions.<br>Group A<br>A dose of 100 µL formulated [$^{14}$C]-Cypermethrin at a nominal concentration of 500 g/L corresponding to 5000 µg/cm$^2$. Each rat received a nominal radioactive dose of 250 kBq.<br>Group B<br>A dose of 100 µL formulated [$^{14}$C]-Cypermethrin at a nominal concentration of 0.25 mg/L corresponding to 0.25 µg/cm$^2$. Each rat received a nominal radioactive dose of 12 kBq. | |
| 3.3.5 Size of test site | 10 cm² - defined by 'O' ring | |
| 3.3.6 Exposure period | Experimental start 26 January 2009<br>Treatment AT1 - 24 h 27- 28 January 2009<br>Treatment AT2 - 72 h 27- 30 January 2009<br>Treatment AT3 - 216 h 27 January – 5 February 2009<br><br>Treatment BT1 - 24 h 3 - 4 February 2009<br>Treatment BT2 - 72 h 3 - 6 February 2009<br>Treatment BT3 - 216 h 3 – 12 February 2009<br>Experimental termination 16 February 2009<br><br>The dermal absorption of [14C]-Cypermethrin was investigated in two groups of male rats consisting of 3 subgroups of 4 animals each.<br><br>A (high dose): undiluted concentrate<br>B (low dose): representative field dilution<br>Details of the test groups are presented in Table IIIA 6.2-2 below. | |
| 3.3.7 Sampling time | 8h and 24h after initiation of skin contact, then at 24 hour intervals up to 208 hours after completion of exposure | |

| **Section A6.2**<br>**Annex Point IIA VI.6.2** | **Percutaneous absorption (in vivo test)**<br>**In-vivo dermal absorption in rat dermis** | |
|---|---|---|
| 3.3.8 Samples | Skin washing – 8h after application.<br>Urine and faeces collected at 24 h intervals to termination<br>At sacrifice the following samples were collected:-<br>- 'O'-ring + protective device<br>- Skin wash at sacrifice<br>- Surface tape strips (stratum corneum), individually sampled<br>- Application site (tape stripped)<br>- Skin (non treated area)<br>- Whole Blood<br>- Plasma<br>- Gastro Intestinal Tract<br>- Residual carcass<br>Cage washes collected at end of the collection phase.<br>The dislodgeable dose was determined after an exposure period of 8 hours. The covering tape from the protective device was removed and retained for analysis. The unabsorbed test substance was removed from the application site by washing 8 times with a mild lukewarm soap solution using cotton swabs. The moist skin area was dried with 2 additional cotton swabs.<br><br>A fresh cover was applied by fitting an extra strong tape around the body and protective device. By doing this, interference of the animal with the exposure chamber and the risk of losing the 'O'-ring and protective device was prevented. The possibility of oral ingestion of the test substance was also minimised (in practice, no parts of the protective device were subsequently found in the faeces).<br><br>Skin stripping – following sacrifice the skin was shaved (the skin shavings were retained for analysis) and then the skin stripping process was initiated.<br><br>Skin stripping involved the application of adhesive tape for circa 5 seconds before the tape was carefully removed against the direction of hair growth. Skin stripping was continued until a 'shiny' appearance of the viable epidermis (stratum lucidum) was evident. This indicated that the stratum corneum had been removed. Usually 12-15 tape strips are sufficient to remove the stratum corneum but for calculation purposes a total of 20 tape strips were taken. Any hair removed was combined with the first tape strip and digested in 1.5 M KOH/20% ethanol in water. | |

| **Section A6.2**<br>**Annex Point IIA VI.6.2** | **Percutaneous absorption (in vivo test)**<br>**In-vivo dermal absorption in rat dermis** | |
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| | Radioactivity was determined in all samples collected.<br>Dose preparations: Samples of the dose preparations were taken just prior to and directly after and diluted with ethanol. Duplicate aliquots were added directly to liquid scintillant (Ultima Gold™) and radioactivity measured by liquid scintillation counting. The average radioactive concentration was used to calculate the total amount of radioactivity administered with the respective dose preparation.<br>Liquid specimens: Aliquots of urine, cage wash, skin wash, plasma, extracts of cover and 'O'-ring were added directly to liquid scintillant (Ultima Gold™) and the radioactivity measured by liquid scintillation counting.<br>Skin strips: Individual skin strips were added directly to liquid scintillant (Hionic Fluor™). After 24 hours extraction the radioactivity was measured by liquid scintillation counting.<br>Faeces, whole blood: Radioactivity in faeces and whole blood was determined by combustion of air-dried subsamples.<br>**Combustion analysis**<br>Samples were combusted using a Packard Sample Oxidizer System 387. The CO2 formed was mixed with Carbosorb™ and Permafluor E™ scintillation fluid and radioactivity measured by liquid scintillation counting.<br>**Digestion**<br>The application site, non-treated skin, GI-tract with contents, hair and residual carcass were digested with 2 to 4 parts of 1.5 M KOH in 20% ethanol and homogenized using an Ultra-turrax. Duplicate aliquots (0.5-1.0 g) were mixed with liquid scintillant (Hionic Fluor™) and radioactivity measured by liquid scintillation counting.<br>**Radioactivity measurements**<br>Radioactivity in all samples was determined by Liquid Scintillation Counting (LSC) on a Tri-Carb 3100TR liquid scintillation counter using QuantaSmart™ software. All counts were converted to DPM using tSIE/AEC (transformed Spectral Index of external standards coupled to Automatic Efficiency Correction. All radio-assays of biological specimens, except skin strips, were performed in duplicate.<br>**Background samples**<br>Background values were measured with each sample sequence using the respective scintillation mixture without any samples. Limits of quantification for radioactivity in samples were calculated using the background + 3x standard deviation of the counting error of the background, as limit of quantification. | |

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|---|---|---|
| | **4 RESULTS AND DISCUSSION** | |
| **4.1 Toxic effects, clinical signs** | The animals were checked for appearance and behaviour during acclimatisation, treatment and at each sampling point.<br>No treatment or dose related signs of toxicity or unusual behaviour were observed.<br>The body weights in the high dose groups showed a slight reduction between the study start and sacrifice timepoints. There was little variation in bodyweight among the low dose animals over the same timeframe. | |

| **Section A6.2**<br><br>**Annex Point IIA VI.6.2** | **Percutaneous absorption (in vivo test)**<br>**In-vivo dermal absorption in rat dermis** | |
|---|---|---|
| **4.2 Retention at application site** | Mean recovery of radioactivity ranged from 99.9% to 100.2.% in the high dose groups and from 99.8 to 105.6% in the low dose groups.<br><br>Individual data expressed as µg Parent Compound equivalents per gram tissue (PCE/g tissue) are presented in the report.<br><br>The amount of radioactivity in the skin strips from the application site is presented graphically in Figures 1 and 2. The amounts remaining in the application site are presented graphically in Figure 3<br><br>**Skin stripping**<br><br>After the 8 h exposure and 16 h post application period – (i.e. at 24 hours after initial of dosing), 7.93% of the dose remained on/in the skin in the high dose group and and 8.53% remained in skin for the low dose group.<br><br>The skin stripping process (total of 20 tapestrips used to removethe *stratum corneum* and reveal the *stratum lucidum*) showed that most radioactivity at the application site was in the stratum corneum, and therefore the potential for absorption was limited.<br><br>The total amount of radioactivity in all 20 skin strips after 24 h was 7.11% and 7.41% of the administered dose for the high and low dose group, respectively.<br><br>In the high dose group the mean radioactivity in total skin strips was 6.73% and 5.03% after 72 h and 216 h, respectively.<br><br>For the low dose, residual radioactivity in total skin strips was 6.54% and 4.34% after 72 h and 216 h, respectively.<br><br>At both dose levels skin stripping revealed that most radioactivity in the *stratum corneum* was concentrated in the upper layer and/or hair at 144 h after the start of application.<br><br>**Stripped skin**<br><br>In the high dose group 0.82% of the administered dose remained in the stripped skin after 24 h (recovered from skin wash at 8 h and 16 h post application) and radioactivity slightly increased to 1.23% after 216 hours.<br><br>In the low dose group, 1.12% of applied radioactivity remained in the stripped skin after 24 h (8 h exposure +16 hours post exposure) but this decreased to 0.20% after 216 h. The levels of radioactivity found in control skin were negligible at all dose levels and time groups.<br><br>**Plasma, whole blood and carcass**<br><br>The highest radioactivity (0.59% of the dose) was found in the residual carcass of animals receiving the low dose, 24 h after the start of application. The levels of radioactivity in plasma and whole blood were close to or below the limit of detection in all time points in the low dose groups and all far below 1 ng/g. | |

| **Section A6.2**<br>**Annex Point IIA VI.6.2** | **Percutaneous absorption (in vivo test)**<br>**In-vivo dermal absorption in rat dermis** | |
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| **4.3 Recovery of labelled compound** | The dose preparations were analysed for stability and purity. The results are tabulated in Table IIIA 6.2-2 below.<br>Mean recovery of radioactivity ranged from 99.9% to 100.2.% in the high dose groups and from 99.8 to 105.6%. in the low dose groups. [One rat, Animal No. 22, had a low skin wash value and a high amount of radioactivity in/on the ring and cover. As a consequence this animal was exposed for much longer to the test substance, leading to a high absorption value. The results for this animal were therefore omitted for calculation of mean and standard deviation.] | |
| **4.4 Percutaneous absorption** | **Absorption, excretion**<br>Individual data are presented in the report for radioactivity in excreta, skin wash and tissues and summarised in Table IIIA 6.2-6 below.<br>After administration of the high dose 0.98±0.82% of the dose was absorbed within 24 h. The total absorption increased to 1.09±0.15% at 72 h and 3.44±1.05% at 216 h after dosing.<br>For the low dose 5.62±1.48 % of the dose was absorbed within 24 h. The total absorption increased slightly to 8.63±0.92% at 72 h and 11.03±1.66 at 216 h after dosing.<br>The urinary excretion accounted for 2.26% and 8.18% of the high and low dose absorption, respectively, within 216 h.<br>Recovery from faeces accounted for 0.38% and 2.08% of the high and low dose absorption, respectively, within 216 h.<br>The bulk of the applied Cypermethrin applied to the skin was removed by washing. Radioactivity in the skin wash was 84-91% of the applied radioactivity for the low dose. In the high dose ca 91% of the applied radioactivity was found in the skin wash.<br>The amounts of radioactivity found in the 'O'-ring and cover after application was considerable (6-8%)at the high dose but lower (2.0-2.5%) in the low dose.<br>The average systemic dose over the last 24 h in each time group is presented in Table IIIA 6.2-7 below. The systemic dose was calculated as total radioactivity absorbed up to sacrifice minus total radioactivity excreted in the previous days (the cage wash included only in the 24 h time group). The results presented in the table demonstrate a low systemic dose (ca 1%) in the time groups of the high dose and a steady decrease from 5.6% after 24 h to ca 1% after 216 h in the low dose. | |
| | **5 APPLICANT'S SUMMARY AND CONCLUSION** | |
| **5.1 Materials and methods** | This study was designed to examine the percutaneous absorption of [$^{14}$C]-Cypermethrin, formulated as Cypermethrin 500 g a.i./L EC, *in vivo* using rats. Cypermethrin was tested at two target concentrations: the emulsifiable concentrate (500 g a.i./L) and a representative field dilution (25 mg a.i./L).<br>The study objectives were to determine the extent of percutaneous absorption of the compound related radioactivity, its permeation through the skin into the body, and its elimination *via* excreta following a | |

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contact time of 8 hours.

The bioavailability of the residues remaining in/on skin after washing of the application site and the kinetics of percutaneous absorption were estimated at 3 time points, 24, 72 and 216 hours after the beginning of the 8 hour exposure time. Furthermore, the distribution of radioactivity between the upper skin layer (*stratum corneum*) and the rest of the skin was estimated.

Cypermethrin was applied at two dose levels - 500 g a.i./L and 25 mg a.i./L equating to the suspension concentrate and a representative field dilution. Subgroups were dosed for each assessment timepoint (24, 72 and 216 h). For each dose level and time point, 4 male Wistar rats were used. The rats were shaved prior to dosing, following suitable acclimation and determination that the skin was undamaged.

**Test groups**

| Sub group | Nom. conc. | Nominal dose[1] | No. of rats | Exposure + post exposure time[2] | In-life period |
|---|---|---|---|---|---|
| AT1 | 500 g/L | 5000 µg.cm$^{-2}$ | 4 | 8 h + 16 h | 24 h |
| AT2 | 500 g/L | 5000 µg.cm$^{-2}$ | 4 | 8 h + 64 h | 72 h |
| AT3 | 500 g/L | 5000 µg.cm$^{-2}$ | 4 | 8 h + 208 h | 216 h |
| BT1 | 25 mg/L | 0.25 µg.cm$^{-2}$ | 4 | 8 h + 16 h | 24 h |
| BT2 | 25 mg/L | 0.25 µg.cm$^{-2}$ | 4 | 8 h + 64 h | 72 h |
| BT3 | 25 mg/L | 0.25 µg.cm$^{-2}$ | 4 | 8 h + 208 h | 216 h |

100 µL of the dose preparations was applied to 10 cm$^2$ of clipped skin. The test site was defined by an 'O'ring stuck to the dermis and covered with a semi-permeable dressing.

Group A
A dose of 100 µL formulated [$^{14}$C]-Cypermethrin at a nominal concentration of 500 g/L corresponding to 5000 µg/cm$^2$. Each rat received a nominal radioactive dose of 250 kBq.

Group B
A dose of 100 µL formulated [$^{14}$C]-Cypermethrin at a nominal concentration of 0.25 mg/L corresponding to 0.25 µg/cm$^2$. Each rat received a nominal radioactive dose of 12 kBq.

The treated skin was washed at the completion of the 8 hour exposure period. Excreta samples were collected at 24 h intervals up to the scheduled sacrifice time (24, 72 or 216 hours). At sacrifice the following samples were collected -

'O'-ring + protective device; skin wash; surface tape strips (stratum corneum), individually sampled; application site (tape stripped); skin (non treated area); whole blood; plasma; gastrointestinal tract; residual carcass. The cage washings at the end of the collection period were also retained.

Verification of the amount of radioactivity applied to the skin was completed before application and analysis of recovery radioactivity was assessed based on the various collected samples and a mass balance completed.

Determination of the degree of dislodgeable dose and also the amount of

| **Section A6.2**<br>**Annex Point IIA VI.6.2** | **Percutaneous absorption (in vivo test)**<br>**In-vivo dermal absorption in rat dermis** | |
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| | dose retained in the skin was investigated using multiple washing to remove non-absorbed material at the end of the exposure period and the use of tape strips (20 in total) to remove the *stratum corneum* sequentially until the *stratum lucidum* was revealed.<br><br>Samples were analysed for radioactivity by means of liquid scintillation counting following appropriate preparation of the samples. | |
| **5.2 Results and discussion** | ***Absorption (systemically available)***<br><br>Total absorption was assessed from recoveries of radioactivity in urine and faecal samples, cage wash, tissues, GI tract and residual carcass as 0.98±0.82% of the high dose absorbed within 24 h. The total absorption increased to 1.09±0.15% at 72 h and to 3.44±1.05% at 216 h after dosing.<br>For the low dose 5.62±1.48% of the dose was absorbed within 24 h. The total absorption increased to 8.63±0.92% at 72 h and to 11.03±1.66 at 216 h after dosing.<br><br>The systemic dose per day showed a low systemic dose (ca 1%) in the time groups of the high dose and a steady decrease from 5.6% after 24 h to ca 1% after 216 h in the low dose.<br><br>The highest amount of radioactivity, 0.59% of the administered dose, was the residual amount found in the low dose carcases, at the 24 h sacrifice. Levels in blood and plasma were close to or below the limit of detection. The low dose values were considerably less than 1 ng/g tissue.<br><br>***Skin strips***<br><br>In the high dose mean radioactivity in skin strips was 7.11% after 24 h, 6.73% after 72 hours and 5.03% at 216 h after dosing.<br><br>For the low dose, residual radioactivity in skin strips was 7.41% after 24 h, 6.54% after 72 hours and 4.34% after 216 h.<br><br>These values include all 20 tape strip amounts. Due to the persistence of absorption over the extended observation period and in accordance with current guidance on use of tape strip data (eg EFSA or MOTA 3), the total amount of absorbed material or biologically available material for absorption was adjusted to exclude the amounts recovered at each time point in the first two tape strips – see discussion below.<br><br>At both dose levels skin stripping revealed that most radioactivity in the stratum corneum was concentrated in the upper layer and/or hair at 216 h after the start of application and therefore the potential for further absorption was limited. | |

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|---|---|---|
| | ***Stripped skin***<br><br>Radioactivity in the dermis (stripped skin) increased from 0.82% at 24 h to 1.23% at 216 h after application in the high dose group. For the low dose the radioactivity decreased from 1.12% at 24 h to 0.20% at 216 h after application.<br><br>In the high dose group sacrificed 16 hours after the 8 hour exposure, 0.82% of administered dose was found in the skin (after washing the dislodgeable amount and removing the stratum corneum by tape stripping ). This residual amount in the skin increased slightly to 1.23% of administered dose when assessed at 216 hours.<br><br>For the low dose groups, radioactivity in stripped skin was 1.12% of administered dose after 24 h and decreased to just 0.20% by 216 hours.<br><br>***Elimination***<br><br>The majority of the radioactivity associated with administered Cypermethrin was removed by washing. The dislodgeable, non – absorbed radioactivity in the skin wash was 84-91% of the applied radioactivity for the low dose and in the high dose circa 91% of the applied radioactivity was removed in the skin wash.<br><br>Additionally significant amounts of radioactivity were associated with the 'O'-ring and cover - 6-8% in the high dose groupand slightly lower, 2.0-2.5%, in the low dose.<br><br>Urinary excretion accounted for 2.26% and 8.18% of the high and low dose, respectively, measured over the 216 h assessment period.<br><br>Urinary excretion at 24 and 72 hours equated to 0.28% and 0.71% of the administered dose in the high dose group and 2.74% and 6.43% in the low dose.<br><br>Faecal excretion accounted for small amounts of radioactivity - 0.38% and 2.08% of the administered high and low doses respectively, within 216 h. High dose faecal excretion at 24 or 72h was 0.07% and 0.15%. The values forthe low dose group were 0.38% and 1.12% respectively. | |

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|---|---|---|
| **Discussion** | Based on the results of this study and taking account of the Manual of Technical Agreements (MOTA3) Biocides Technical Meeting, 24 Feb, 2010, the RMS considered *in vivo* percutaneous absorption study of an EC formulation in rats (concentrate 500g ai/L; spray dilution 25 mg a.i./L = agriculture field dilution, 8 h exposure, post exposure times 24h, 72h, 216h), revealed dermal absorption values, including residual skin (stripped skin) at 24h, 72h or 216 h after dosing of -<br><br>Concentrate: 1.8, 2.1, 4.7%<br><br>Spray dilution: 6.5, 9.2, 11.2%. This would assume excluding all of the tape stripping recovered radioactivity.<br><br>The dermal absorption values including residual skin and **all 20** tapestrips at 24h, 72h or 216 h after dosing:<br><br>Concentrate: 8.9, 8.9, 9.7%<br><br>Spray dilution: 13.94, 15.8, 15.6%.<br><br>The draft 'Guidance on Dermal Absorption' recently circulated by EFSA Panel on Plant Protection Products and their Residues (PPR) seeks to clarify the use of data obtained by skin stripping. In the current study, a total of 20 strips were used and the total dermal absorption values calculated above reflect either the inclusion of results from all strips or from none. Neither position is thought to accurately reflect the role of the stratum corneum as a partial barrier to dermal absorption and a means of removal of non-absorbable material.<br><br>The EFSA Guidance reflects the conclusions of MOTA3 in the Biocides Technical Meeting. Where there is no evidence for continuing absorption at the end of the observation period, it is justified to exclude the tape strip data entirely. In cases where more than 75% of absorption occurs within half of the observation period, then tape strip data may alsobe excluded unless only pooled data are available. In this study the absorption after 72 hours was less than 75% of the total after 216 h and so inclusion of some tape strip data is considered appropriate.<br><br>The RMS has suggested not including values equivalent to the top 25% of the stratum corneum, but in the absence of data relating to the percent of corneum removed by each strip and without an assumption of a linear dispersal of radioactivity throughout the stratum corneum it is not possible to estimate this value from the available data. The total absorption has therefore been recalculated to include the total absorbed plus any radioactivity recovered from stripped skin and also a value for the percent of applied dose recovered in tape strips 3 to 20, excluding the amounts in strips 1 and 2. These values are presented in Table IIIA 6.2-8.<br><br>There is general agreement, reflected in both MOTA3 (4.1.1 Q2) and the most recent EFSA Guidance on Dermal Absorption (point 5.1.1) that the amount of dose removed by the initial two tapestrips represents material that will not become biologically available and that practically and pragmatically these data can be excluded for the total absorption calculation.<br><br>Based on these assumptions the dermal absorption values for cypermethrin including total absorbed, residual skin absorption and 18 tapestrips (first two excluded) at 24 h, 72 h or 216 h after dosing are:<br><br>**Concentrate: 6.7, 7.0, 7.6%**<br><br>**Spray dilution: 12.5, 13.6, 12.7%.** | |

| **Section A6.2**<br>**Annex Point IIA VI.6.2** | **Percutaneous absorption (in vivo test)**<br>**In-vivo dermal absorption in rat dermis** | |
|---|---|---|
| **5.3 Conclusion** | Cypermethrin formulated as 500 EC (500 g a.i./L) and a representative field dilution (25 mg a.i./L) were applied to rats for an 8 h exposure period.<br><br>The majority of radioactivity associated with Cypermethrin applied to the skin was removed by washing at the end of the exposure period. Radioactivity recovered in the skin wash accounted for 84-91% of the applied radioactivity for the low dose and in the high dose circa 91% of the applied radioactivity was recovered in the skin wash.<br><br>Additionally the amounts of radioactivity found in the 'O'-ring and cover after application was considerable (6-8%) in the high dose but much lower (2.0-2.5%) in the low dose.<br><br>Within 24h, 0.98±0.82% of the high dose was absorbed. The total absorption increased to 1.09±0.15% at 72 h and 3.44±1.05% at 216 h after dosing. For the low dose 5.62±1.48 % of the dose was absorbed within 24 h. The total absorption increased to 8.63±0.92% at 72 h and 11.03±1.66 at 216 h after dosing.<br><br>In the high dose mean radioactivity in thetotal (20) skin strips was 7.11% after 24 h and 5.03% at 216 h after dosing. For the low dose, residual radioactivity in twenty skin strips was 7.41% after 24 h and 4.34% after 216 h. Most radioactivity in the stratum corneum was concentrated in the upper layer and/or hair at 216 h after the start of application and therefore the potential for absorption was limited.<br><br>Radioactivity in the dermis (stripped skin) slightly increased from 0.82% at 24 h to 1.23% at 216 h after application in the high dose group. For the low dose the radioactivity decreased from 1.12% at 24 h to 0.20% at 216 h after application.<br><br>The urinary excretion accounted for 2.26% and 8.18% of the high and low dose, respectively, within 216 h. The faeces accounted for 0.38% and 2.08% of the high and low dose, respectively, within 216 h.<br><br>Penetration of Cypermethrin through rat skin at a typical concentration (25 mg/L) used in the field is 11% and 3.4% for the formulated concentrate (500 g/L).<br><br>There is general agreement, reflected in both MOTA3 (4.1.1 Q2) and the most recent EFSA Guidance on Dermal Absorption (point 5.1.1) that the amount of dose removed by the initial two tapestrips represents material that will not become biologically available and that practically and pragmatically these data can be excluded for the total absorption calculation.<br><br>Based on these assumptions the **revised** dermal absorption values for cypermethrin including total absorbed, residual skin absorption and 18 tapestrips (first two excluded) at 24 h, 72 h or 216 h after dosing are:<br><br>**Concentrate: 6.7, 7.0, 7.6%**<br><br>**Spray dilution: 12.5, 13.6, 12.7%.** | |
| 5.3.1 Reliability | 1 | |
| 5.3.2 Deficiencies | No | |
| | | |

| **Section A6.2**<br>**Annex Point IIA VI.6.2** | **Percutaneous absorption (in vivo test)**<br>**In-vivo dermal absorption in rat dermis** | |
|---|---|---|
| | **Evaluation by Competent Authorities** | |
| | **EVALUATION BY RAPPORTEUR MEMBER STATE** | |
| **Date** | January, 2011. | |
| **Materials and Methods** | The applicant's version is acceptable. | |
| **Results and discussion** | The applicant's version is adopted. | |
| **Conclusion** | The applicant's version is adopted. | |
| **Reliability** | 1 | |
| **Acceptability** | acceptable | |
| **Remarks** | | |
| | **COMMENTS FROM ...** | |
| **Date** | | |
| **Materials and Methods** | | |
| **Results and discussion** | | |
| **Conclusion** | | |
| **Reliability** | | |
| **Acceptability** | | |
| **Remarks** | | |

**Table IIIA 6.2-1 Dose preparations**

| Group | Dose | [$^{14}$C]-Cypermethrin | Cypermethrin 500 g/L EC | Cypermethrin 500 g/L EC placebo | Nominal concentration | Nominal radioactivity concentration |
|---|---|---|---|---|---|---|
| A | High dose | 7.50 MBq (~ 1.57 mg) | 3.0 mL | - | 500 g.L$^{-1}$ | 2.5 MBq.mL$^{-1}$ |
| B | Low dose | 5.97 MBq (~1.25 mg) | - | 50 mL* | 25 mg.L$^{-1}$ | 120 kBq.mL$^{-1}$ |

- diluted 20000 times with demineralised water
-

**Table IIIA 6.2-2 Characteristics of dose preparations**

| Dose preparation (day of use) | Concentration | | Specific Activity ** (kBq/mg a.i.) | Purity HPLC (%)*** |
|---|---|---|---|---|
| | DPM/g ± CV | mg a.i.*/g | | |
| AT1, AT2, AT3 (27 January 2009) | 1.436E+08 ± 0.19% | 478.77 | 4.999 | 96.9-96.8 |
| BT1, BT2, BT3 (3 February 2009) | 6.913E+06 ± 4.42% | 0.0241 | 4780 | 97.1-96.9 |

* Active ingredient.

** Calculated by total radioactivity measured divided by total labelled and non-labelled Cypermethrin.

*** The purity before and directly after dosing is given

**Table IIIA 6.2-3 Overview of test groups**

| Subgroup | Nominal concentration | Nominal dose[1] | Number of animals | Exposure/post exposure time[2] | In-life period |
|---|---|---|---|---|---|
| AT1 | 500 g.L$^{-1}$ | 5000 µg.cm$^{-2}$ | 4 | 8 h + 16 h | 24 h |
| AT2 | 500 g.L$^{-1}$ | 5000 µg.cm$^{-2}$ | 4 | 8 h + 64 h | 72 h |
| AT3 | 500 g.L$^{-1}$ | 5000 µg.cm$^{-2}$ | 4 | 8 h + 208 h | 216 h |
| BT1 | 25 mg.L$^{-1}$ | 0.25 µg.cm$^{-2}$ | 4 | 8 h + 16 h | 24 h |
| BT2 | 25 mg.L$^{-1}$ | 0.25 µg.cm$^{-2}$ | 4 | 8 h + 64 h | 72 h |
| BT3 | 25 mg.L$^{-1}$ | 0.25 µg.cm$^{-2}$ | 4 | 8 h + 208 h | 216 h |

[1] 100 µL of the dose preparations was applied to 10 cm$^2$ of clipped skin.

[2] post-exposure monitoring after washing skin with a neutral aqueous detergent to remove non-absorbed material.

| **Calculations performed on experimental data** | |
|---|---|
| All calculations were performed with electronic devices with varying degrees of soft- and hardware dependent floating-point precision. Numerical values in this report are frequently rounded to a smaller number of digits than were used in the actual calculation to increase readability and to indicate the approximate precision of the reported results. Minor differences in results obtained from calculations with such rounded values in comparison to those obtained with higher precision values are well within the limits of the experimental accuracy and therefore of no practical concern. Data resulting in 0.0 or 0.00 after rounding are reported as < 0.1 and < 0.01, respectively. Non-rounded data were used for calculating means and standard deviations. | |
| **Activity dosed per animal:** | TC = dose * TCG - dose rest<br>where TC = total DPM administered to the animal<br>dose = weight of the administered dose in g<br>TCG = radioactivity of the administered dose in DPM/g<br>dose rest = total DPM in the dose rest |
| **Radioactivity calculated per gram of sample:** | XC(1, 2) = (XD(1, 2) – Blank) / XW(1, 2)<br>XC = (XC(1) + XC(2)) / 2<br>where XC = concentration in DPM per g of sample X<br>XD = DPM counted for aliquot of sample X<br>XW = weight of aliquot of sample X<br>(1, 2) = various aliquots<br>Blank = appropriate blank value in DPM |
| **Radioactivity calculated in µg parent compound equivalents per g of tissue:** | XPC = XC / SA<br>where XPC = concentration in µg parent compound equivalents per g of sample X<br>XC = concentration in DPM per g of sample X<br>SA = specific activity of the test substance in DPM per µg of parent compound |
| **Radioactivity calculated as percentage of the dose administered:** | X% = (XC * X / TC) * 100<br>where X% = percentage radioactivity of the dose administered in sample X<br>XC = concentration in DPM per g of sample X<br>X = total weight of sample X in g<br>TC = total DPM administered to the animal<br>All results were rounded to two digits. If the result was lower than 0.005% the result is presented as < 0.01. |
| **Limit of Quantification** (µg/g): | LQ(PCE) = Blank/XW * SA<br>where LQ(PCE) = Limit of determination of sample in µg Parent Compound Equivalents/g<br>Blank = appropriate blank value in DPM<br>XW = Weight of aliquot of sample X in g<br>SA = Specific Activity of the test substance (DPM/µg Parent Compound Equivalents) |

| | | |
|---|---|---|
| | **Absorbed fraction** | µg equivalents in carcass plus sum of the tissues and organs |
| | **(systemically available)** | prepared plus blood, urine, cage wash, and faeces |
| | **Systemic dose** | % of radioactivity absorbed in the body (excluding application site) up to sacrifice minus % of radioactivity excreted in the previous days |
| | **Amounts on/in skin** | µg equivalents on/in skin and tape strips |
| | **Not absorbed fraction** | µg equivalents in the skin wash, and on the protective device |

**Table IIIA 6.2-4 Summary table of systemic dose**

| Systemic dose (expressed as % of administered dose) | | | | | | |
|---|---|---|---|---|---|---|
| Nominal dose | A: 5000 µg.cm$^{-2}$ | | | B: 0.25 µg.cm$^{-2}$ | | |
| Time group | T1 | T2 | T3 | T1 | T2 | T3 |
| period | 0-24 h | 48-72 h | 192-216 h | 0-24 h | 48-72 h | 192-216 h |
| Systemic | 0.98 | 0.49 | 1.04 | 5.62 | 2.21 | 1.06 |
| dose | ± 0.82 | ± 0.18 | ± 0.72 | ± 1.48 | ± 0.19 | ± 0.20 |

**Table IIIA 6.2-5 Concentration and distribution of Cypermethrin in tissue**

| Average values (n =4) expressed as µg PCE/ g of tissue* | | | | | | |
|---|---|---|---|---|---|---|
| Nominal dose | A: 5000 µg.cm$^{-2}$ | | | B: 0.25 µg.cm$^{-2}$ | | |
| Time | T1 | T2 | T3 | T1 | T2 | T3 |
| group | 24 h | 72 h | 216 h | 0-24 h | 71 h | 216 h |
| Plasma | 0.182 | 0.130 | 0.190 | 0.00007 | 0.00005 | < 0.00002 |
| Whole Blood | 0.068 | 0.135 | 0.102 | 0.00015 | 0.00008 | 0.00004 |
| Residual Carcass | 2.436 | 0.418 | 2.463 | 0.00007 | 0.00006 | <0.00005 |

*µg Parent compound Equivalents per gram of tissue

Figure 1

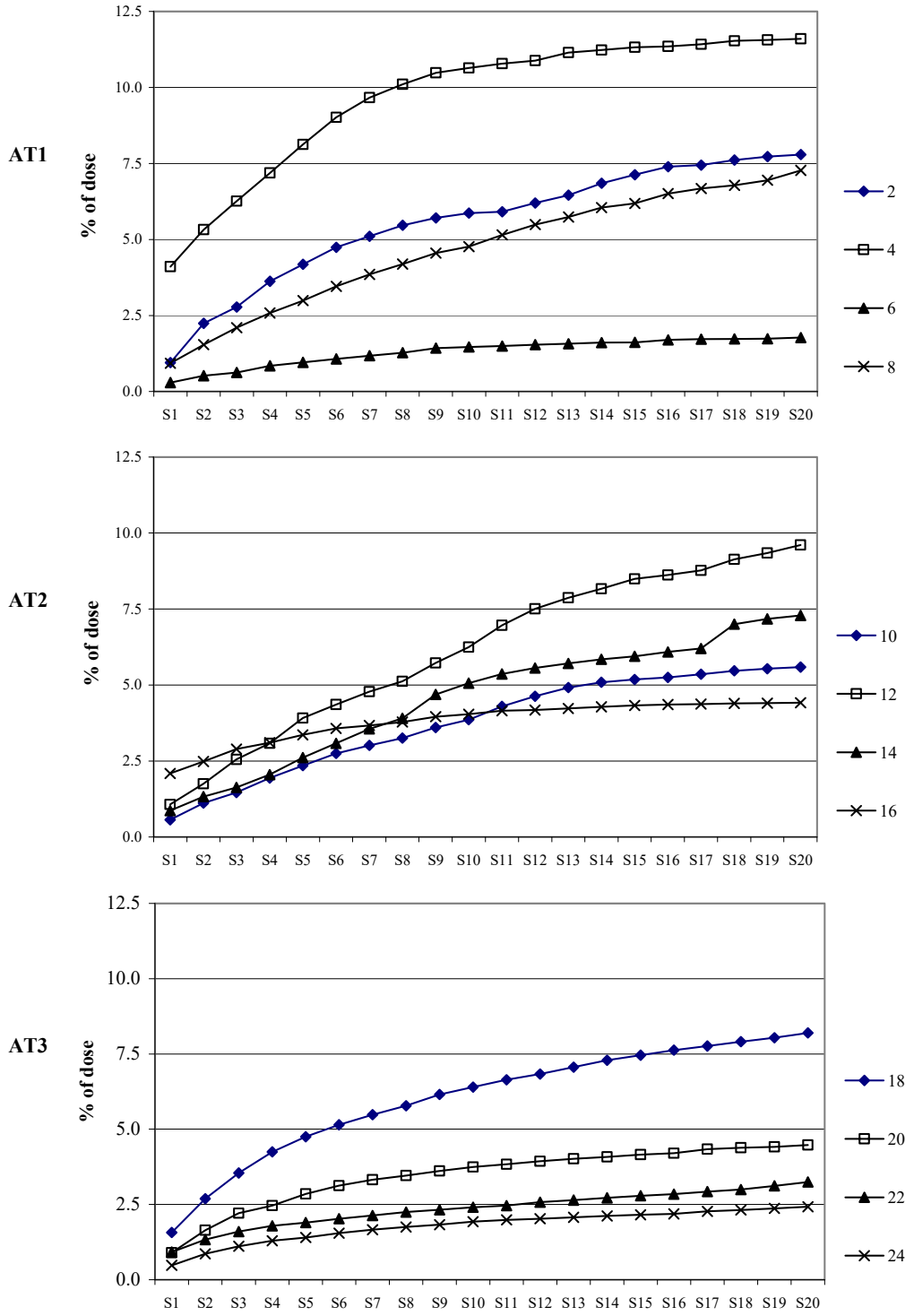



*Figure A4* *Skin stripping of the application site of male Wistar rats,16 h (AT1), 64 h (AT2) or 216 h (AT3) after removal of the unabsorbed dose following dermal application of a high dose (A: 5000 µg/cm$^2$) of [$^{14}$C]-Cypermethrin*
*Expressed as cumulative % of the dose stripped.*
*Strip number S1-S20, the legend show the individual rat numbers*

Figure 2

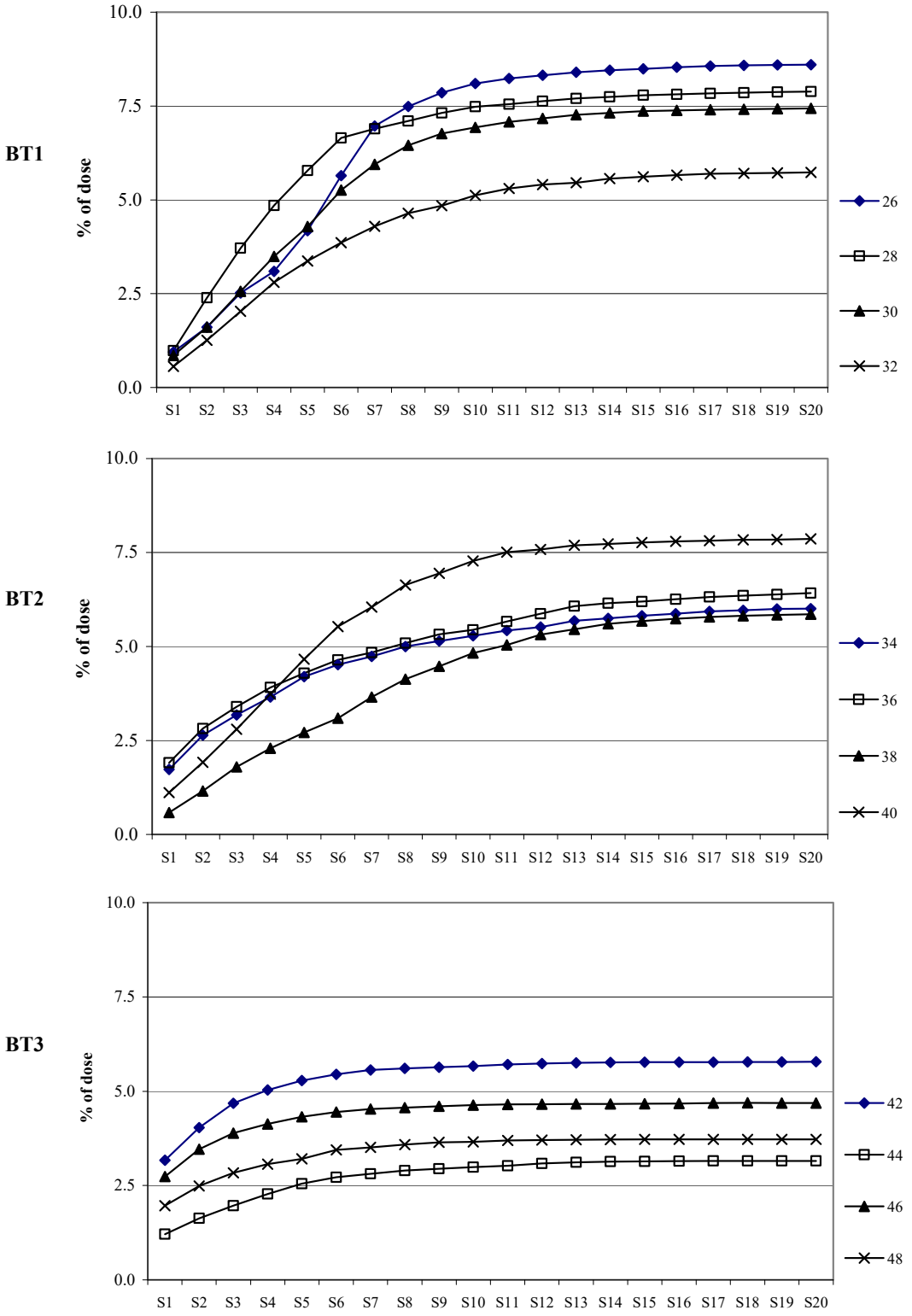
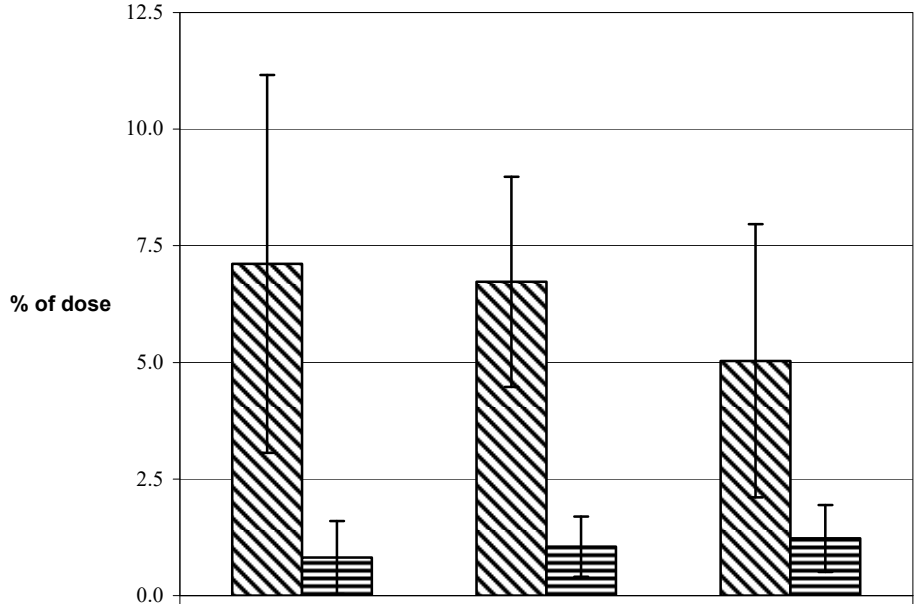



*Figure A5* *Skin stripping of the application site of male Wistar rats,16 h (BT1), 64 h (BT2) or* 216 h (BT3) after removal of the unabsorbed dose following dermal application of a low dose (B: 0.25 µg/cm$^2$) of [$^{14}$C]-Cypermethrin. Expressed as cumulative % of the dose stripped. *Strip number S1-S20, the legend show the individual rat numbers.*

Figure 3

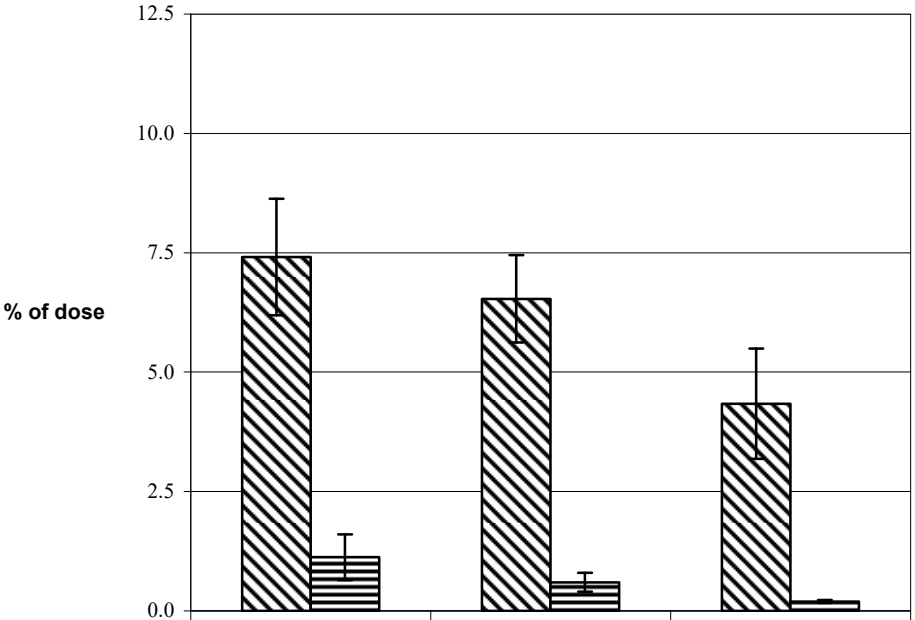
*Distribution of radioactivity in skin strips and stripped skin in the application site of male Wistar rats 16 h (T1), 36 h (T2) or 136 h (T3)after removal of the unabsorbed dose following dermal application of a high dose (A: 5000 µg/cm$^2$) or a low dose (B: 0.25µg/cm$^2$ ) of [$^{14}$C]-Cypermethrin.*
*Expressed as % of the dose (average ± SD).*

**A**




| | AT1 | AT2 | AT3 |
|---|---|---|---|
| Skin strips | 7.11 | 6.73 | 5.03 |
| Stripped skin | 0.82 | 1.05 | 1.23 |

**B**




| | BT1 | BT2 | BT3 |
|---|---|---|---|
| Skin strips | 7.41 | 6.54 | 4.34 |
| Stripped skin | 1.12 | 0.60 | 0.20 |

**Table IIIA 6.2-6 Summary table of absorption and excretion**

| Average values (n=4) expressed as % of the dose applied | | | | | | |
|---|---|---|---|---|---|---|
| Nominal dose | A: 5000 µg.cm$^{-2}$ | | | B: 0.25 µg.cm$^{-2}$ | | |
| Time group | T1 24 h | T2 72 h | T3 216 h | T1 24 h | T2 72 h | T3 216 h |
| Urine (total) | 0.28 | 0.71 | 2.26 | 2.74 | 6.43 | 8.18 |
| Faeces (total) | 0.07 | 0.15 | 0.38 | 0.38 | 1.12 | 2.08 |
| Cage wash | 0.01 | 0.01 | 0.09 | 0.18 | 0.24 | 0.12 |
| Tissues** | < 0.01 | < 0.01 | < 0.01 | 0.06 | 0.04 | 0.05 |
| GI-tract | 0.13 | 0.07 | 0.15 | 1.66 | 0.29 | 0.17 |
| Carcass | 0.49 | 0.15 | 0.56 | 0.59 | 0.51 | < 0.43 |
| **Absorbed** | **0.98** | **1.09** | **3.44** | **5.62** | **8.63** | **11.03** |
| Skin strips | 7.11 | 6.73 | 5.03 | 7.41 | 6.54 | 4.34 |
| Stripped skin | 0.82 | 1.05 | 1.23 | 1.12 | 0.60 | 0.20 |
| **Total skin** | **7.93** | **7.78** | **6.26** | **8.53** | **7.13** | **4.54** |
| Total skinwash | 82.75 | 84.80 | 82.18 | 89.53 | 85.43 | 81.76 |
| O-ring/cover | 8.38 | 6.26 | 8.34 | 1.95 | 2.46 | 2.42 |
| **Not absorbed** | **91.13** | **91.06** | **90.52** | **91.48** | **87.89** | **84.18** |
| **Recovery** | **100.04** | **99.93** | **100.22** | **105.64** | **103.66** | **99.75** |

** : Terminal blood obtained by exsanguination + control skin

**Table IIIA 6.2-7 Summary table of systemic dose**

| Systemic dose (expressed as % of administered dose) | | | | | | |
|---|---|---|---|---|---|---|
| Nominal dose | A: 5000 µg.cm$^{-2}$ | | | B: 0.25 µg.cm$^{-2}$ | | |
| Time group period | T1 0-24 h | T2 48-72 h | T3 192-216 h | T1 0-24 h | T2 48-72 h | T3 192-216 h |
| Systemic dose | 0.98 ± 0.82 | 0.49 ± 0.18 | 1.04 ± 0.72 | 5.62 ± 1.48 | 2.21 ± 0.19 | 1.06 ± 0.20 |

**Table IIIA 6.2-8 Revised estimate of dermal absorption (not including amounts recovered in first two tape strips)**

| Average values (n=4) expressed as % of the dose applied | | | | | | |
|---|---|---|---|---|---|---|
| Nominal dose | A: 5000 µg.cm$^{-2}$ | | | B: 0.25 µg.cm$^{-2}$ | | |
| Time group | T1<br>24 h | T2<br>72 h | T3<br>216 h | T1<br>24 h | T2<br>72 h | T3<br>216 h |
| **Absorbed (Sum of urine, faeces, cage wash, tissues, GI tract and residual carcass** | **0.98** | **1.09** | **3.44** | **5.62** | **8.63** | **11.03** |
| Skin strips All 20 included | 7.11 | 6.73 | 5.03 | 7.41 | 6.54 | 4.34 |
| Stripped skin | 0.82 | 1.05 | 1.23 | 1.12 | 0.60 | 0.20 |
| **Total skin** | **7.93** | **7.78** | **6.26** | **8.53** | **7.13** | **4.54** |
| Skin strips, first two strips removed | 4.89 | 4.83 | 2.89 | 5.77 | 4.38 | 1.48 |
| Stripped skin | 0.82 | 1.05 | 1.23 | 1.12 | 0.60 | 0.20 |
| **Total skin** | **5.70** | **5.90** | **4.12** | **6.89** | **4.98** | **1.68** |
| **Total absorption** | **6.68** | **6.99** | **7.56** | **12.51** | **13.61** | **12.71** |