Permethrin 5% w/w Dermal Cream

2.4 Nonclinical Overview

Module Prepared by: [redacted]

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Contents

Abbreviations

3PBA	3-phenoxybenzoic acid
3PBACID	3-phenoxybenzoic acid
3PBALD	3-phenoxy-benzaldehyde
4′-OH-PBA	4′OH-phenoxybenzoic acid
ACGIH	American Conference of Governmental Industrial Hygienists
ACh	acetylcholine
AChE	acetylcholinesterase
AIDS	acquired immune deficiency syndrome
ANS	Panels on Food Additives and Nutrient Sources Added to Food
AUC	under the plasma concentration-time curve
BBB	blood-brain barrier
BHT	butylated hydroxytoluene
bw	body weight
CEPA	Californian Environmental Protection Agency
CL_{int}	intrinsic clearance
C_{max}	peak plasma concentration
CNS	central nervous system
CYP	cytochromes p450
DCCA	3-(2,2 dichlorovinyl)-2,2-dimethyl-(1-cyclopropane) carboxylic acid
DEET	N,N-diethyl-m-toluamide
DMBA	7,12-dimethylbenz[a]anthracene
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
ED_{50}	effective dose (50%)
F	bioavailability
FCC	Food Chemicals Codex
FDA	Food and Drug Administration
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
GDs	gestation days
GMS	glycerol monostearate
GRAS	generally recognized as safe
H_{12}-HTX	perhydrohistrionicotoxin
IARC	International Agency for Research on Cancer
IH	in-house
IID	Inactive Ingredient Database
IM	intermuscular
IP	intraperitoneal
IPCS	International Programme on Chemical Safety

IUPAC	International Union of Pure and Applied Chemistry
IV	intravenous
LD_{50}	lethal dose (50%)
LOEL	lowest-observed-effect-level
LoQ	limit of quantitation
METP	permethrin metabolites
MRT	mean residence time
MSDS	Material Safety Data Sheet
NaOH	sodium hydroxide
NF-kB	nuclear factor-kappaB
NOEL	no-effect-level
NRC	National Research Council
Nrf2	nuclear factor (erythroid-derived 2)-like 2
NS	no significant difference
PAA	poly(acrylic acid)
PBPK	physiologically based pharmacokinetic model
PEG	polyethylene glycol
Ph.Eur.	European Pharmacopoeia
PO	per os
ppb	parts per billion
ppt	parts per trillion
PVA	polyvinylalcohol
RADS	reactive airways dysfunction syndrome
RLD	Reference Listed Drug
SC	subcutaneous
SCE	sister chromatid exchange
SCO	saponified coconut oil
SEM	standard error of mean
$t_{½\alpha}$	distribution half-lives
$t_{½\beta}$	elimination half-lives
T_{max}	time to C_{max}
UK	United Kingdom
US	United States
USP	United States Pharmacopeia
UV	ultraviolet
WHO	World Health Organization

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2.4.1 Overview of the Nonclinical Testing Strategy

Permethrin [(±)-3-phenoxybenzyl 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-carboxylate] belongs to the family of synthetic chemicals called pyrethroids. Permethrin is widely used as an insecticide, acaricide, and insect repellent; and is considered as first-line treatment for scabies. Permethrin is also effective against lice infesting humans including crab lice. Permethrin is on the World Health Organization's (WHO) List of Essential Medicines, the most important medications needed in a basic health system (. Permethrin 5% w/w Dermal Cream is indicated for the treatment of scabies in adults and children > 2 months of age, and crab lice in adults.

Scabies is a parasitosis caused by the mite *Sarcoptes scabiei* var *hominis* (;). Crusted scabies is a more severe form of the disease *n,* . Crusted scabies is also called "Norwegian scabies" because the condition was first described in Norway in the mid-19th century. Norwegian scabies is different from traditional scabies due to an impaired immune response to the mites allowing for the infestation of an individual with hundreds of thousands of the mites. Crusted scabies is observed most frequently in the elderly, mentally or physically disabled, and in patients with acquired immune deficiency syndrome (AIDS), lymphoma, or other conditions that decrease the effectiveness of the immune response ( ). In these cases, spread of infection may occur during brief contact or via contaminated objects. Because the mite is very small and is usually not directly visible, diagnosis is based on the signs and symptoms. The standard diagnostic technique consists of mites' identification by microscopic examination of scales obtained by skin scraping ( ). The disease manifestations are mediated through inflammatory and hypersensitivity reactions to mites and mite products). Infestation is accompanied by intense itching, papular rash, and emotional disturbance from the concept of arthropod infestation (

Infestation begins when pregnant female mites are transferred from the skin of an infested person to the skin of an uninfested person ( ). Following infestation, the adult female mite travels on the skin surface at the rate of about 2.5 cm per minute seeking a burrow site. Finding a suitable location, the female mite burrows into superficial layers of the skin, forming a slightly elevated narrow tunnel where she deposits 2 to 3 eggs daily during her 4 to 6 week life span. The eggs progress through larval and nymphal stages to form adults in 10 to 17 days. The adults migrate to the skin surface and mate. The males die quickly, while the females penetrate the skin and repeat the cycle. In order to complete its life cycle, the mite requires human skin and is unable to survive off the host at room temperature for more than 3 to 4 days.

Effective scabies control requires treatment of affected patients, their close contacts, and environmental fomites ([redacted]). Delayed or missed diagnosis, improper application of medication, inadequate treatment, or poor compliance may cause difficulties. Although the methodological quality of trials vary ([redacted]), a meta-analysis suggests that topical permethrin is the most effective against mites ([redacted]). Treatment with most scabicidal medications calls for treating with an initial dose and re-treating 7 days later; however, the biological basis for when it is optimal to re-treat has not been documented.

The crab louse (*Pthirus pubis*, also called pubic louse) is an insect that is an ectoparasite of humans, feeding exclusively on blood ([redacted] . Crab lice are adapted to a sedentary life style on pubic hair, and sometimes on eyelashes and body hair, not often leaving the infested body. They are usually transmitted during sexual contact, and have been associated with other sexually transmitted diseases [redacted] All lice infestations are diagnosed by identification of live adult lice, and viable eggs (nits) on the hair shafts in the specific body regions giving them their names ([redacted]). Empty egg cases attached to hair shafts are not diagnostic of an active infection.

Permethrin is available for topical use as a cream or lotion. It is indicated for the treatment and prevention in exposed individuals of head lice and treatment of scabies ([redacted], [redacted] . Permethrin formulations include a prescription-only 5% strength for scabies and an over-the-counter 1% strength for lice. Permethrin acts on the nerve cell membrane to disrupt the sodium channel current by which the polarization of the membrane is regulated ([redacted]). Delayed repolarization and paralysis of the pests leads to death.

Permethrin, the active substance is of well-established use, recognised as safe and effective, has high therapeutic margin and no particular concern in terms of toxicity. The excipients in Permethrin 5% Cream of Encube Ethicals Private Limited, India are approved and established agents in widespread use in the pharmaceutical manufacturing industry.

This nonclinical overview has been prepared to review the relevant preclinical pharmacodynamic, pharmacokinetic and safety/tolerability aspects of using permethrin as active ingredient in Permethrin 5% w/w Dermal Cream. All pharmacodynamic, pharmacokinetic and toxicological studies described in this Nonclinical Overview were retrieved from publicly available literature.

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2.4.2 Pharmacology

2.4.2.1 Pharmacology and Mode of Action (Primary Pharmacodynamics)

Pyrethroids can be classified into two types based on the signs of toxicity to mammals ([redacted]) and cockroaches ([redacted] : Type I and Type II compounds. 1R- *cis*- and 1R- *trans*-permethrin belong to Type I. Permethrin is a neurotoxin; it interacts with a fraction of the voltage-dependent sodium channels in excitable membranes that produce a prolongation of the inward sodium current during excitation, in which the channels remain open much longer than normal [redacted]). Membrane depolarization might also occur, resulting in enhanced neurotransmitter release and eventually blockage of excitation. Although postsynaptic neurotransmitter responses can be suppressed by pyrethroids, doses must be higher than those that produce effects on sodium channels ([redacted]). Electrophysiological recordings from dosed cockroaches reveal trains of cercal sensory spikes and, sometimes, spike trains from the cercal motor nerves and the central nervous system. The signs of poisoning caused by Type I pyrethroid compounds are restlessness, incoordination, hyperactivity, prostration, and paralysis ([redacted]).

The effects of seven different pyrethroid insecticides on the lateral-line sense organ and on peripheral nerves of the clawed frog, *Xenopus laevis* were investigated by means of electrophysiological methods. The results show the two classes of pyrethroid can be clearly distinguished ([redacted] Permethrin induced short trains of nerve impulses in the lateral-line sense organ. In peripheral nerve branches, it induced a depolarizing after potential and repetitive firing. In sensory fibres, the effects of permethrin on the nerve action potential are more pronounced compared to its effects on motor fibres. The sodium channel in the nerve membrane is the major target site of permethrin.

1RS- *cis*-permethrin and 1RS- *trans*-permethrin cause tremor (known as T-syndrome) when injected intravenously (IV) into rats at a dose level of more than 270 mg/kg body weight ([redacted]). The onset of the T-syndrome is usually rapid. Rats suffering from T-syndrome exhibit aggressive sparring behaviour and increased sensitivity to external stimuli. This is followed by the appearance of a slight tremor, which gradually becomes more severe and finally reaches a state of prostration and vigorous whole body tremor. The core temperature is markedly increased during poisoning; this may result from the excessive muscular activity associated with tremor.

The major site of action of permethrin (*cis*, *trans*, and technical grade) and deltamethrin, representatives of the non-cyano- and cyano-containing classes, respectively, of synthetic pyrethroids, on the mammalian nervous system were examined in mice ([redacted]). ED_{50} values for the ability of both types of pyrethroids to cause prostration and loss of righting

reflex were estimated following either IV or intracerebroventricular injections. The comparative potencies of the four pyrethroids (deltamethrin > *cis*-permethrin > technical grade permethrin > *trans*-permethrin) were the same following either route of administration. All four compounds tested showed a much greater potency (> 200-fold for deltamethrin, *cis*-permethrin, and technical grade permethrin, and 85-fold for *trans*-permethrin) after intracerebroventricular administration than after IV administration. In addition, the poisoning symptoms seen following direct central injection were almost identical to those obtained with peripheral administration. These results suggest that poisoning from both classes of pyrethroids in mammals is due predominantly to central mechanisms.

Pyrethroid containing products including permethrin were tested ([redacted]). S*arcoptes scabiei* var suis mites were collected from experimentally-infected pigs. For each test, 20 live mites of all motile stages were placed in a plastic Petri dish and sprayed uniformly by each product. Control mites were sprayed by distilled water. The study was performed in triplicate under room conditions and the mites were inspected under a stereomicroscope at intervals (5, 10, 15, 20, 25, 30, 40, 50, 60 min, 2, 3, 4, 5 and 24 h) after exposure to the products. The median survival time was 50 ± 30.4 min and 120 ± 309 min when mites were exposed to permethrin 4 %, and permethrin 0.6%.

The efficacy of a 65% permethrin spot-on formulation against the dog louse, *Trichodectes canis* de Greer 1778, was studied ([redacted]). Fourteen dogs naturally infested with *T. canis* were evenly and randomly allocated to treatment with 65% permethrin administered at the label dose rate of 1 or 2 ml per dog or to an untreated control group. Louse counts were performed for each dog by gently back-combing the hair at six designated anatomic sites (head, tail, belly, each side, and an 8-cm strip the length of the body on the back), and lice were counted without removal on Days 0 (pretreatment), 7, 14, 21, and 28. Lice were eliminated from all dogs treated with the 65% permethrin spot-on within 7 days after treatment, and no subsequent reinfestations due to hatching of eggs were observed during the 28-day evaluation period. Untreated control dogs were subsequently treated with the 65% permethrin spot-on after the initial phase was completed and lice populations were evaluated as previously described. All lice were cleared from these dogs by Day 7, and there were no signs of reinfestation. No adverse reactions to treatment were noted during the study.

2.4.2.2 Secondary Pharmacodynamics

The pyrethroid class can modulate the dopaminergic system by up-regulation of dopamine transporter ([redacted]) and marked increase in dopamine turnover ([redacted]). Permethrin is a potent inhibitor of the mitochondrial complex I ([redacted]., [redacted] . Brainstem acetylcholinesterase (AChE) activity significantly increased following treatment with permethrin ([redacted]).

In a one-month open-label controlled prospective clinical study, the tolerance and the efficacy of a combination of imidacloprid (10%) and permethrin (50%) applied topically as a spot-on,

for the treatment of natural canine fly dermatitis due to *Stomxys calcitrans* were evaluated ([redacted]). The combination of imidacloprid and permethrin proved safe and helpful in the management of natural canine fly dermatitis.

2.4.2.3 Safety Pharmacology

2.4.2.3.1 Cardiovascular effects

The possible cardiac toxicity of permethrin metabolites (METP), 3-phenoxy-benzyl alcohol (3PBA), 3-phenoxy-benzaldehyde (3PBALD), and 3-phenoxybenzoic acid (3PBACID) was investigated ([redacted] . Plasma membrane fluidity, polarity, lipid, and protein oxidation were studied in isolated rat heart cells. Results show that perturbation of rat heart plasma membrane fluidity occurs and is due to metabolites of permethrin.

The impact of early life permethrin treatment (1/50 of LD_{50}, from 6th to 21st day of life) was investigated on the development of cardiotoxicity in 500-day-old rats (*Vadhana et al., 2013*). Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) and nuclear factor-kappaB (NF-κB) gene expression, calcium level and heart surface area were chosen as biomarkers of toxicity. A significant decrease in heart surface area was observed in treated rats (296.59 ± 8.09, mm^2) with respect to the control group (320.86 ± 4.93, mm^2). The intracellular calcium influx in heart of early life treated rats increased 4.33-fold compared to the control one. In conclusion, early life pesticide exposure to low doses of permethrin insecticide has long-term consequences leading to cardiac hypotrophy, increased calcium and Nrf2 gene expression levels in old age.

2.4.2.3.2 Nervous system effects

Permethrin is neurotoxic at high doses ([redacted]). It produces a variety of clinical neurotoxic effects in animals such as tremors, salivation, paraesthesia, splayed gait, depressed reflexes, and tiptoe gait; reversible axonal injury occurs at high doses. Peripheral nerve damage has been reported to occur in laboratory animals at near lethal doses of pyrethroids ([redacted]g [redacted]). In an acute dermal toxicity study, rats exposed to permethrin at 2 g/kg demonstrated neurotoxic signs such as tip-toe gait, upward curvature of the spine, and urinary incontinence in some of the exposed animals (*NRC US, 1994*). Swelling, nodal demyelination, and disintegration of the sciatic nerves were observed in rats fed permethrin at 6,000 ppm (300 mg/ kg per day) for 8 days (*CEPA, 1994*). Pyrethroids can affect behaviour patterns. Mice exposed to permethrin at 0.5, 5.0, or 50 mg/kg, PO or 30 or 300 mg/kg dermally displayed an increased activity at the 50 and 300 mg/kg, PO and dermal doses, respectively [redacted]). Pyrethroids, regardless of structure, produce a decrease in motor activity in a variety of test protocols. The range of relative potencies varies more than two orders of magnitude, and thresholds for motor activity were found well below doses that produce overt signs of poisoning. Permethrin impairs schedule-controlled operant responding and induce an increase in acoustic-evoked startle response amplitude in small rodents ([redacted], [redacted] . Small dose of permethrin (15, 30, 60 mg/kg, IP) disrupts well-established, schedule-

controlled behaviour in male and female rats in a schedule- and gender-dependent manner ([redacted]

Early-life exposure at 6 weeks of age to low levels of permethrin (0.05 mg/kg/day) exerts impairments in learning and memory with the effects on neuronal and glial population in adult male mice ([redacted]

2.4.2.3.3 Other effects

Permethrin caused severe alterations in the liver cells of mice reducing the size of the nuclei and causing hydropic degeneration of the hepatocytes, in addition to stimulating the proliferation of Kupffer cells, altered the amount of proteins, polysaccharides, lipids, and vacuoles in the cytoplasm of the hepatocytes and congested the hepatic capillaries (*Roma et al., 2012*). As for the spleen of the treated mice, no alterations were observed in the morphology in relation to the control group, what would suggest that the spleen would continue performing its functions, without suffering morphological alterations even in the presence of the toxic agent. Permethrin crosses the blood–brain barrier (BBB) and exerts effects on dopaminergic system, contributing to the burden of oxidative stress in Parkinson's disease through several pathways [redacted]).

2.4.2.4 Pharmacodynamic Drug Interactions

Synergic interaction between pesticides has been widely documented, however, the physiological mechanisms by which an insecticide synergizes another remains unclear. Toxicological and electrophysiological studies were carried out on two susceptible pest species (the mosquito *Culex quinquefasciatus* and the cockroach *Periplaneta americana*) to explore the physiological process involved in pyrethroid and carbamate interactions ([redacted]., [redacted] The addition of a sub-lethal concentration of nicotine significantly increased the toxicity of permethrin and propoxur at the lower range of the dose–mortality regression lines, suggesting the manifestation of important physiological disruptions at synaptic level. The effects of both permethrin and propoxur were studied on the cercal afferent giant-interneuron synapses in the terminal abdominal ganglion of the cockroach *P. americana*. Permethrin and propoxur increased drastically the ACh concentration within the synaptic cleft, which thereby stimulated a negative feedback of ACh release. Atropine, a muscarinic receptor antagonist, reversed the effect of permethrin and propoxur mixtures. This demonstrates the implication of the presynaptic muscarinic receptors in the negative feedback regulation process and in synergism. Based on these findings, a cascade of molecular events explains the occurrence of synergistic effects between pyrethroid and carbamate on many susceptible insects including *C. quinquefasciatus*, a mosquito of medical importance.

The interaction of a series of pyrethroid insecticides with the Na^+ channels in myelinated nerve fibres of the clawed frog, *Xenopus laevis*, was investigated using the voltage clamp technique ([redacted]). Out of 11 pyrethroids 9 insecticidally active compounds induce a

slowly decaying Na^+ tail current on termination of a step depolarization, whereas the Na^+ current during depolarization was hardly affected. These tail currents are most readily explained by a selective reduction of the rate of closing of the activation gate in a fraction of the Na^+ channels that have opened during depolarization. The rate of decay of the Na^+ tail current varies considerably with pyrethroid structure. After alpha-cyano pyrethroids, the decay is at least one order of magnitude slower than after non-cyano pyrethroids. The decay always follows a single-exponential time course and is reversibly slowed when the temperature is lowered from 25 to 0 degrees C. Arrhenius plots in this temperature range are linear. These results indicate that the relaxation of the activation gate in pyrethroid-affected Na^+ channels is governed by an apparent first order, unimolecular process and that the rate of relaxation is limited by a single energy barrier. Application of transition state theory shows that after alpha-cyano pyrethroids this energy barrier is 9.6 kJ/mol higher than after non-cyano pyrethroids. Differences in rate of decay of the Na^+ tail current account for the reported differences in repetitive nerve activity induced by various pyrethroids. In addition, the effect of temperature on the rate of decay explains the increase in repetitive activity with cooling.

The interactions of natural pyrethrins and 9 pyrethroids with the nicotinic acetylcholine (ACh) receptor/channel complex of Torpedo electronic organ membranes were studied ([redacted]). None reduced ^{3}H-ACh binding to the receptor sites, but all inhibited ^{3}H-labeled perhydrohistrionicotoxin (^{3}H-H_{12}-HTX) binding to the channel sites in presence of carbamylcholine. Allethrin inhibited binding noncompetitively, but ^{3}H-labeled imipramine binding competitively, suggesting that allethrin binds to the receptor's channel sites that bind imipramine. The pyrethroids were divided into 2 types according to their action: type A, which included allethrin, was more potent in inhibiting ^{3}H-H_{12}-HTX binding and acted more rapidly. Type B, which included permethrin, was less potent and their potency increased slowly with time. The high affinities that several pyrethroids have for this nicotinic ACh receptor suggest that pyrethroids may have a synaptic site of action in addition to their well-known effects on the axonal channels.

The effects of pyrethroids were studied on phosphoinositide breakdown in guinea pig synaptoneurosomes ([redacted]). Similar to other agents that activate voltage-dependent sodium channels, Type I and Type II pyrethroids stimulated phosphoinositide breakdown. Type II pyrethroids, like deltamethrin and fenvalerate, were more potent and, at least for deltamethrin, more efficacious than Type I pyrethroids, like allethrin, resmethrin and permethrin. The effects of Type II pyrethroids could be partially inhibited by the sodium channel blocker tetrodotoxin. The effects of allethrin and resmethrin were not affected by 5 µM tetrodotoxin. Stimulation of phosphoinositide breakdown by fenvalerate was additive to the stimulation elicited by the receptor agonists carbamylcholine and norepinephrine, but not to the stimulation elicited by sodium channel agents (batrachotoxin, scorpion venom and pumiliotoxin B). Stimulation by allethrin was not additive to the stimulation elicited by either receptor agonists or sodium channel agents. A submaximal concentration of allethrin, a Type I

pyrethroid, did not greatly affect the dose-dependent stimulation elicited by a Type II pyrethroid, deltamethrin, while a higher concentration of allethrin prevented further stimulation by Type II pyrethroids. A local anesthetic, dibucaine, which inhibits sodium channel activation, inhibited phosphoinositide breakdown induced by Type II, but not by Type I pyrethroids, except at higher concentrations. Thus, Type II pyrethroids appear to stimulate phosphoinositide breakdown in synaptoneurosomes in a manner analogous to other sodium channel agents, while Type I pyrethroids elicit phosphoinositide breakdown by a different mechanism, probably not involving sodium channels.

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2.4.3 Pharmacokinetics

2.4.3.1 Absorption

Oral (PO) absorption of permethrin was investigated in rats given permethrin dissolved in dimethyl sulfoxide (DMSO) at 1.6-4.8 mg/kg and was estimated to be about 70% for the *cis/trans* (35:65) isomer mix ([redacted]). Only 3-6% of the dose was detected in faeces as unmetabolized and, presumably, unabsorbed permethrin, suggesting that actual absorption might be higher than 70%. The bioavailability of permethrin was estimated to be 60% by comparing the "area under the curve" (AUC) for permethrin in blood after gavage with the AUC after IV injection ([redacted] That low estimate could be due to first-pass biotransformation of permethrin by the liver following absorption. The mean plasma concentrations of permethrin after IV and PO administration of single 46 and 460 mg/kg permethrin doses are shown in Fig. 1.

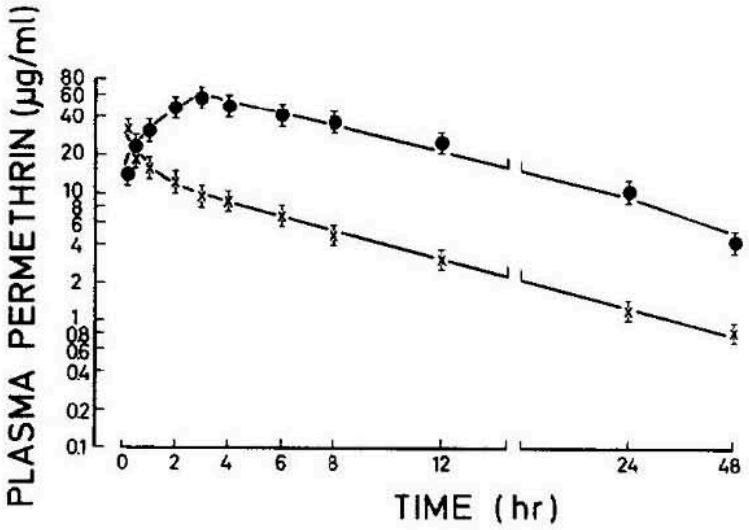

Figure 1 Plasma permethrin concentrations vs time in rats following a single 460 mg/kg oral (●) and 46 mg/kg intravenous (**x**) dose. Data represent mean + SEM for eight rats per data point.

Values for the pharmacokinetic parameters calculated from the mean concentration-time curve are shown in Table 1.

Table 1. Pharmacokinetic parameters for permethrin in rats after a single (460 mg/kg) PO and (46 mg/kg) IV dose

Parameter	IV	PO
C_{max} (L/hr)	-	49.46
T_{max} (h)	-	3.52
AUC (mh.h/L)	159	965
MRT (h)	11.19	49.46
$t_{½\alpha}$	0.46	4.85
$t_{½ß}$	8.67	12.37
F (%)	-	60.69

C_{max} - peak plasma concentration; T_{max} - time to C_{max}; AUC - under the plasma concentration-time curve; MRT - mean residence time; $t_{½\alpha}$ - distribution half-lives; $t_{½ß}$ – elimination half-lives; F - bioavailability.

The percutaneous absorption of permethrin is generally lower in humans than in other mammalian species ([redacted]). An acetone solution of permethrin was applied to the shaved backs of mice to investigate dermal penetration. Permethrin penetrated the skin of mice most rapidly, the $T_{½}$ being 5.9 min. Within 5 min, 40% of the permethrin had moved from the site of application.

The percutaneous absorption of *cis/trans* permethrin was determined in rhesus monkeys and rats ([redacted]). Radiolabelled permethrin was applied to either the forehead or the forearm of rhesus monkeys or to the mid-lumbosacral region of the rat. Urine was collected for 7-14 days, and the recovered radioactivity in the urine was compared with that obtained after intermuscular (IM) injection to determine the percent absorption. In monkeys, the forehead was more permeable than the forearm (14-28%, forehead; 5-12%, forearm). The forehead is likely to be more absorbent than the forearms because the forehead is more glabrous (smooth and without hairs) and more highly vascularized than the forearms. The absorption of permethrin applied to the backs of rats was significantly greater than the forehead or the forearm in the monkey (rat, 43-46%). The interspecies difference supports the finding of higher permeability of rat skin demonstrated with other pharmaceutical compounds and underscores the importance of using caution in extrapolating the results of pesticide dermal absorption studies in nonprimate species to humans.

2.4.3.2 Distribution

Pyrethroids are lipophilic molecules that generally undergo rapid absorption and distribution following ingestion by mammals ([redacted]). Unless isolated in lipid depots, they are quickly metabolized and eliminated from the body. Permethrin persists longer in fat than in other tissues when measured in chickens, rats, goats, and cows.

Permethrin is a chiral molecule presenting two forms, the *cis* and the *trans* isomers. *In vitro* studies indicate a metabolic interaction between the *trans* and *cis* isomers of permethrin. A physiologically based pharmacokinetic model (PBPK) was adapted and calibrated for *trans*- and *cis*-permethrin separately in Sprague–Dawley rats ([redacted]). The model also describes the toxicokinetics of three urinary metabolites, *cis*- and *trans*-3-(2,2 dichlorovinyl)-2,2-dimethyl-(1-cyclopropane) carboxylic acid (*cis*- and *trans*-DCCA); 3-phenoxybenzoic acid (3-PBA) and 4′OH-phenoxybenzoic acid (4′-OH-PBA). The schematic of the models of *cis*- and *trans*-permethrin and their metabolites following oral exposure are represented in Fig. 2.

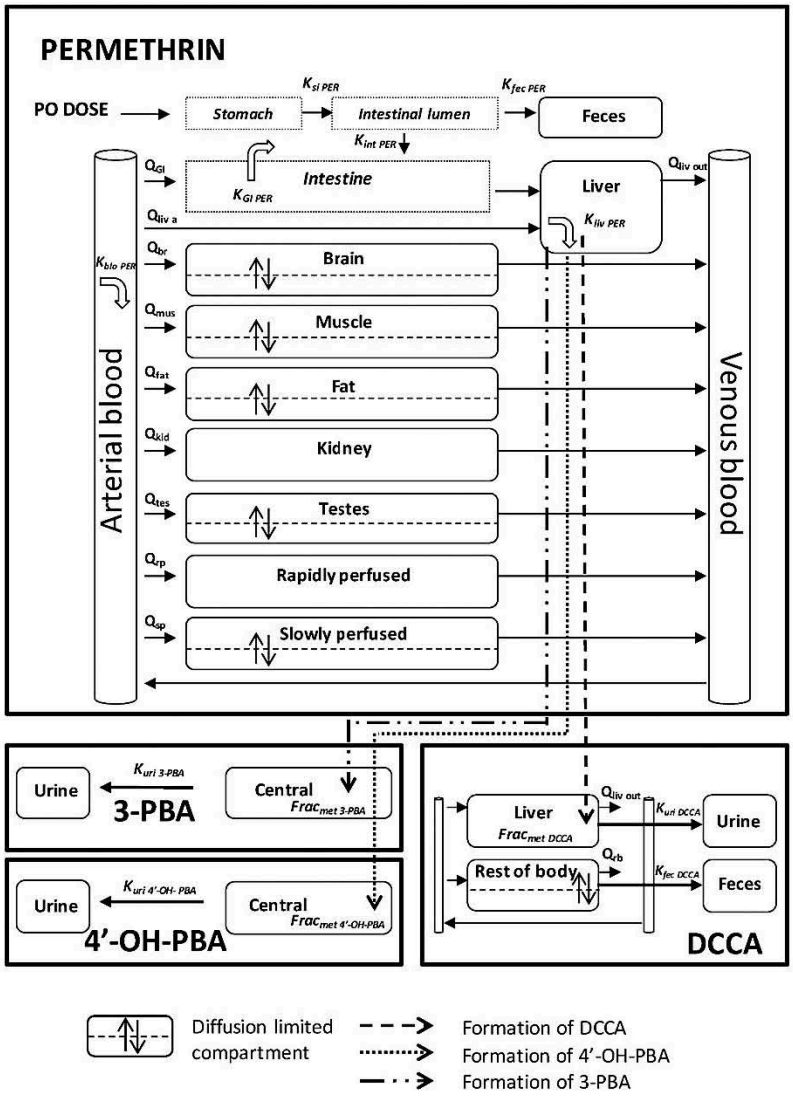

Figure 2. PBPK model of *cis*- and *trans*-permethrin and their metabolites *cis*- and *trans*-DCCA, 3-PBA and 4′-OH-PBA with oral exposure. Distribution in brain, muscle, fat, testes and slowly perfused tissues of parent compounds and in rest of body of DCCA are limited by the diffusion and distribution in other organs by the flow. 3-PBA, DCCA, 4′-OH-PBA are formed in liver ([redacted]).

The estimated concentrations of permethrin isomers using the PBPK model were in good correlation with the observed concentrations for all the fluids and organs (Fig. 3).

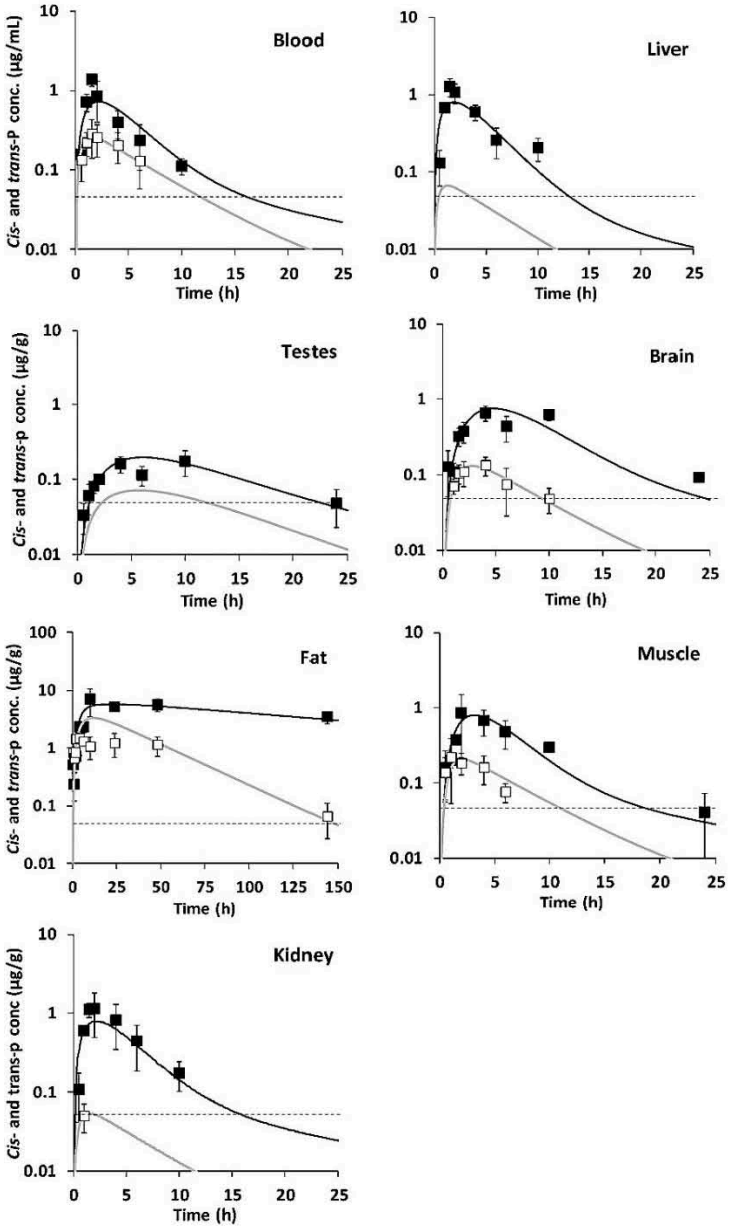

Figure 3. Measured concentrations of *cis*-permethrin (*cis*-p) (■) and *trans*-permethrin (*trans*-p) (□) and toxicokinetic profiles estimated with the PBPK model (solid lines) of *cis*-permethrin (▬) and *trans*-permethrin (▬) in blood, liver, kidney, brain, testes, muscle and fat in rats after an exposure to 25mg/kg of *cis*- or *trans*-permethrin. The gray dotted line (---) stands for the limit of quantitation (LoQ) of permethrin.

Cis-permethrin and *trans*-permethrin was quantified in faeces, kidney, mammary gland, fat and placenta in pregnant rats with oral dosing of 50 mg/kg permethrin ([redacted]). The

maximal plasma and tissue concentrations occurred at 6 h after the administration. The highest concentrations were observed in fat and mammary glands. Despite the greater proportion of the trans isomer in the administered dose, *cis*-permethrin concentrations were higher than *trans*-permethrin. *cis*-Permethrin and *trans*-permethrin were detected in foetal blood and tissues confirming the placental transfer. Foetal concentrations were lower than maternal concentrations for blood and brain. In contrast, foetal liver concentrations were higher than corresponding maternal values.

The influence of immaturity and sex on plasma and target organ dosimetry of each of the insecticide's two isomers, *cis*- and *trans*-permethrin was determined [redacted]). Postnatal day 15, 21 and 90 (adult) Sprague-Dawley rats were orally administered a graduated series of doses of *cis*- and *trans*-permethrin in corn oil. Levels of trans-permethrin decreased relatively rapidly, despite administration of relatively high doses. Concentrations of each isomer in plasma, brain, and other tissues monitored were inversely proportional to the animals' age. The youngest pups exhibited 4-fold higher plasma and brain AUCs than did adults. Little difference was observed in the toxicokinetics of cis- or trans-permethrin between adult male and female rats, other than higher initial plasma and liver *cis*-permethrin levels in females. Greater permeability of the immature BBB to *cis*- and *trans*-permethrin may contribute to the increased susceptibility of pre-weanling rodents to the insecticides ([redacted]).

The toxicokinetics of permethrin taken PO at 460 mg/kg or taken IV at 46 mg/kg was studied in male Sprague-Dawley rats ([redacted]). The elimination half-time of permethrin was slower for the hippocampus, medulla oblongata, front cortex, and sciatic nerve (16-24 hr) than for plasma (12 hr). Higher amounts of permethrin were also found in those tissues than in plasma, indicating the accumulation of permethrin by nervous tissue. The metabolites of permethrin, m-phenoxy benzoalcohol and m-phenoxy benzoic acid, were detected in plasma and in all selected tissues for 48 hr after dosing.

Studies in Sprague-Dawley rats, in which a variety of pyrethroid insecticides were administered PO, demonstrated that the residues of permethrin in fat and brain were much higher and more persistent with *cis*-permethrin than with *trans*-permethrin (*NRC US, 1994*). Fat and brain concentrations of the *trans* isomer but not the *cis* isomer were greatly elevated on pretreatment with either esterase or oxidase inhibitors.

Studies using human skin fibroblast androgen receptors have demonstrated that nonsteroidal compounds, including permethrin, can interact competitively with human androgen receptors and the sex hormone binding globulin ([redacted] Those studies provide a mechanism by which chronic exposure of humans to pesticides containing nonsteroidal compounds might result in endocrine disturbances relating to androgen action. The competitive binding studies demonstrate that permethrin is a weak binder compared with other agents, such as R1888, a non-metabolizable synthetic androgen.

Binding of deltamethrin, *cis*-permethrin, and *trans*-permethrin to plasma proteins and lipoproteins was linear from 250 to 750 nM in the rat (*Sethi et al, 2019*).

2.4.3.3 Metabolism

The liver is quantitatively the most important site for permethrin biotransformation. The metabolic pathway in animals is similar to that in humans. The two major pathways for metabolism of permethrin are oxidation and hydrolysis ([redacted]). As with other pyrethroids, *trans* isomer metabolism is dominated by hydrolysis and *cis* isomer metabolism is dominated by oxidation. The major pyrethroid hydrolysing esterase is located in mammalian liver microsomes and is probably a carboxyl esterase. The *cis* and *trans* pyrethroid isomers show substrate specificity – the *trans* form being hydrolysed up to 50 times faster than the *cis* form. The presence of a hydrolysis inhibitor should prolong the tissue distribution and retention of permethrin. Oxidation is also an important route of metabolism for pyrethroids, for the *cis* isomers, since they are less likely to be metabolized by hydrolysis ([redacted]). Oxidative reactions occur at the cyclopropane carboxylic acid moiety, at the alcohol moiety, and probably in the proximity of the ester bond so that its cleavage is catalysed. This later process might be very important for *cis* isomers, which are more resistant to hydrolysis. Additionally, oxidation at peripheral sites, while leaving the ester bond intact, affords points at which conjugation reactions occur, leading to biliary and faecal elimination of the esters. The metabolic pathways of permethrin in mammals are shown in Fig. 4.

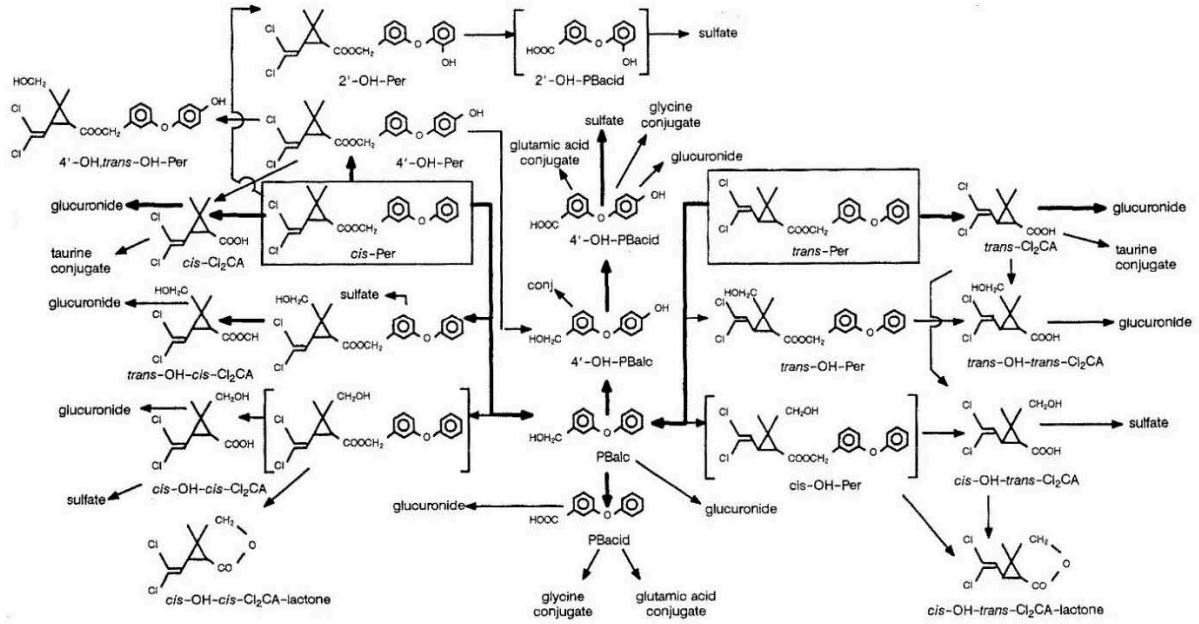

Figure 4. Metabolic pathways of permethrin in mammals ([redacted]).

The impact of incubating a mixture of pyrethroid isomers on the intrinsic clearance (CL_{int}) of the individual isomers was examined using permethrin (40:60, *cis*/*trans*) ([redacted]).

The CL_{int} of *cis*-permethrin incubated either as an individual isomer or in the mixture was 10-fold greater or more in rat than in human hepatic microsomes. *Trans*-permethrin was cleared more quickly by human than rat hepatic microsomes when incubated as an individual isomer. The CL_{int} of *trans*-permethrin was similar between microsomes from both species when incubated in the mixture. However, the CL_{int} of *trans*- permethrin in the mixture decreased by 40% in rat and 64% in human hepatic microsomes. The CL_{int} of total permethrin was approximately 3-fold greater in rat than in human hepatic microsomes. Percentage metabolism of *cis*- and *trans*-permethrin by rat P450 isoforms is shown in Table 2.

Table 2. Percentage metabolism of *cis*- and *trans*-permethrin by human P450 isoforms

Isoform	*cis*-Permethrin	*trans*-Permethrin
CYP1A1	47 ± 8	58.3 ± 7.5
CYP1A2	12.3 ± 4.5	38.3 ± 7.5
CYP2A1	NS	NS
CYP2B1	NS	NS
CYP2C6	83.7 ± 5	88.7 ± 2.1
CYP2C11	77.7 ± 15.9	82 ± 5.3
CYP2C12	NS	NS
CYP2C13	NS	NS
CYP2D1	NS	NS
CYP2D2	NS	NS
CYP3A1	30.3 ± 5.5	NS
CYP3A2	16.7 ± 1.2	13.3 ± 3.5

NS – No significant difference. The percentage metabolism of pyrethroids by rat P450s was assessed by a one-sample t-test. Values that were not significantly different from 0 were labelled NS ($p < 0.05$).

2.4.3.4 Elimination

The pyrethrin metabolites are generally excreted as alcohols, phenols, or carboxylic acids and their glycine, sulphate, or glucuronide conjugates. At least 80 metabolites have been identified from *cis* and *trans*-permethrin in various species and systems ([redacted]).

The 1R *trans*, 1RS *trans*, 1R *cis*, and 1RS *cis* isomers separately labelled in the acid and alcohol moieties were given PO to rats, and metabolites in urine and faeces were identified ([redacted], [redacted]. The results showed that there was no significant metabolic difference between 1R and 1RS isomers, although *cis* permethrin isomers were more likely to undergo oxidative metabolism than the *trans* counterparts. Twelve days after administration, 97-100% of the radioactivity was recovered in urine and faeces. Unchanged permethrin was detected only in the faeces. Radioactivity from the *cis* isomers tended to be retained longer than that from the *trans* isomers and that from the alcohol label longer than that from the acid label. The most striking difference was that only 45-54% of the radiocarbon from the *cis* isomer appeared in

the urine, whereas 81-90% of that from the *trans* isomer appeared in the urine. The more hydrolytically stable *cis* isomer resulted in metabolites that retained the ester bond, and these metabolites were excreted in the faeces, presumably via the bile. The large molecular weight of permethrin (391.29 g/M) suggests that it would be an excellent candidate for biliary excretion. The major metabolites from both isomers were the sulphate and glucuronide conjugates of the phenoxybenzoic acid portion of the molecule and the glucuronide conjugate of the cyclopropane carboxylic acid portion.

2.4.3.5 Pharmacokinetic Drug Interactions

The possibility for enhancement of permethrin transdermal absorption by N,N-diethyl-m-toluamide (DEET) has been reported ([redacted]). Subchronic dermal application of DEET and permethrin to adult rats, alone or in combination, causes diffuse neuronal cell death and cytoskeletal abnormalities in the cerebral cortex and the hippocampus, and Purkinje neuron loss in the cerebellum ([redacted]). These results suggest that DEET used in combination with permethrin might serve to facilitate the absorption of permethrin through the skin.

MODULE 2 OVERALL SUMMARIES

2.4 Nonclinical Overview

2.4.4 Toxicology

2.4.4.1 Single Dose Toxicity

Permethrin is one of the least toxic synthetic pyrethroid insecticides to mammals, especially when compared with the more commonly used insecticides - organochlorine, organophosphorus, and methylcarbamate. The results of acute toxicity tests of permethrin with various animal species are shown in Table 3 ([redacted]).

Table 3. Acute toxicity of permethrin administered to various animal species

Species	Sex[a]	Route[b]	Vehicle	LD_{50} (g/kg bw)
Rat	M	PO	water	2.949
	F	PO	water	> 4.0
	M	PO	DMSO	1.5
	F	PO	DMSO	1.0
	M	PO	corn oil	0.5
	M	PO	corn oil	0.43
	F	PO	corn oil	0.47
	M&F	PO	corn oil	1.2
	M&F	PO	water	1.725
	M	dermal	water	> 5.176
	F	dermal	-	> 4.0
	M	dermal	-	> 2.5
	F	dermal	-	> 2.5
	M&F	dermal	xylene	> 0.75
	M&F	dermal	-	2.0
	M	SC	corn oil	7.8
	F	SC	corn oil	6.6
	M	IP	water	> 3.2
	F	IP	water	> 3.2
Mouse	F	PO	water	> 4.0
	M&F	PO	DMSO	0.25 – 0.5
	M	PO	corn oil	0.65
	F	PO	corn oil	0.54
	M	dermal	-	> 2.5
	F	dermal	-	> 2.5
	M	SC	corn oil	> 10.0
	F	SC	corn oil	10.0
Rabbit	F	PO	water	> 4.0
	F	dermal	-	> 2.0
Guinea-pig	M	PO	water	> 4.0

[a] F – female, M – male; [b] PO – oral, SC – subcutaneous; IP – intraperitoneal

The acute (single dose) oral LD_{50} of permethrin (purity 90.5-97.2% and consisting of mixtures of *cis/trans* isomers in various proportions) in animals (rats, mice, rabbit, and guinea pigs) is in the range of 0.25-10 g/kg, depending on the vehicle used for administration. Permethrin is more toxic when formulated with corn oil, DMSO, and propylene glycol than when in an aqueous suspension (perhaps because of greater solubility of permethrin in organic solvents than in water. Death in animals occurs within 3 days of exposure to permethrin. The *cis/trans* isomeric ratio also appears to affect toxicity, the *cis* isomer being more toxic than the *trans* isomer in animals ([redacted](Table 4).

Table 4. Acute toxicity of permethrin WITH various *cis/trans* isomeric ratios

Permethrin (*cis/trans*)	Species	Sex	Route	LD_{50} (mg/kg)
80:20	Rat	F	PO	396
57:43		F	PO	333
50:50		F	PO	748
40:60		F	PO	630
20:80		F	PO	2800
99:1	Mouse	NA	IP	108
40:60		NA	IP	514
1:99		NA	IP	> 800
99:1		NA	IV	17
40:60		NA	IV	31
1:99		NA	IV	> 135

NA – not available

Clinical signs of toxicity, when evident, occur within 2 h and are associated with central nervous system dysfunctions. Exposure to permethrin is associated with tremors, convulsions, irregular breathing and increased respiratory rates, incoordination, ataxia, hyperactivity, prostration, and paralysis. Other signs that have been reported include hyperexcitability to external stimuli, lacrimation, occasional diarrhoea, defecation, and urinary incontinence. Core body temperature is increased when clinical signs are severe. Signs of toxicity can last up to 3 days after acute exposure. Acute toxicity of permethrin from dermal exposure is lower than that from other routes of exposure in several animal species (see Table 3). No deaths were observed when technical-grade permethrin was applied to the skin of rats at 2 g/kg, but tiptoe gait, upward curvature of the spine, and urinary incontinence were observed in several animals.

2.4.4.2 Repeat Dose Toxicity

Mouse

Alderley Park mice (20 of each sex per group) were fed permethrin in the diet at concentrations of 0, 200, 400, 2,000, or 4,000 mg/kg of diet for 28 days ([redacted]). Mortality, growth, and food consumption were normal in all dose groups. One additional group (80 mg/kg of permethrin for 2 weeks and 10.000 mg/kg for the final 2 weeks) showed weight loss and

poor food consumption when permethrin feeding was begun at 10,000 mg/kg. Mice that were fed permethrin at 2.000 mg/kg or more showed increased liver weight and liver-to-body-weight ratio. Higher weight and organ-to-body-weight ratios were also observed in the heart, kidney, and spleen of male mice in the 10.000 mg/kg dose group. Gross tissue changes were observed in female mice that received 2.000 and 10.000 mg/kg, respectively. Histopathological examination showed regenerating tubules in the renal cortex, hypertrophy of centrilobular hepatocytes with cytoplasmic eosinophilia that were not dose related; these changes were observed in all the treated animals.

In another study, groups of six female mice were administered daily oral doses of permethrin (*cis/trans* ratio, 25:75) in corn oil at 0, 200, 400, 800, or 1,600 mg/kg for 10 consecutive days ([redacted]). Signs of acute toxicity, such as spasm and convulsion, were seen only in the animals of the highest dose group, half of which died after the initial dose. No significant changes were observed in haematology, clinical chemistry, or body weights after 11 doses. The mice treated at 800 and 1600 mg/kg exhibited increased liver weights.

Rat

Long-Evans rats (six of each sex per group) were administered permethrin in the diet for 2 weeks at 0, 27, 54, 108, 216, or 432 mg/kg per day ([redacted]). Rats surviving to term were killed, and various tissues and organs were examined histopathologically. At 432 mg/kg, three of six females died in the first 5 days. Muscle tremors were observed in all surviving animals in the 216- and 432-mg/kg groups. A statistically significant increase was seen among female rats in the liver-to-body-weight ratio. Compound-related histological changes were not observed in any of the tissues or organs examined. The maximum dietary no-effect-level (NOEL) was calculated to be 108 mg/kg per day.

Sprague-Dawley rats (six of each sex per group) were fed permethrin in the diet for 14 days at dose levels of 54, 108, 216, 432, 864, or 1728 mg/kg body weight per day ([redacted]). Rats surviving to term were sacrificed and various organs and tissues were examined histopathologically. At the two highest dose levels, all animals died except one female fed 864 mg/kg. Muscle tremors were noted in all animals at 432 mg/kg, but doses of 216 mg/kg or less caused no toxic signs in either males or females. There was a statistically significant increase in average liver-to-body weight ratios at 432 mg/kg, but compound-related histological changes were not observed in any of the tissues or organs. The maximum NOEL in this study was 216 mg/kg.

Wistar rats (eight of each sex per group) were fed permethrin at 0, 200, 500, 1,000, 2,500, 5,000, or 10,000 mg/kg of diet for 4 weeks ([redacted]). All rats that received the highest dose died within 3 days. Mortality was seen at 5,000 mg/kg, and hyperexcitability was observed in animals that received 2,500 mg/kg. Food consumption and growth decreased in animals in the 5,000 mg/kg group. There was no effect on haematological values, clinical chemistry, or urinalysis except for a reduction in urinary protein excretion in male rats at 5,000

mg/kg. Liver weight and liver-to-body-weight ratios were increased in males at 2,500 mg/kg or greater and in females at 1,000 mg/kg or greater.

The reversibility of hepatic changes was studied in Wistar rats following short-term dietary administration of permethrin ([redacted]). Female Wistar rats (48 rats per group) were fed permethrin at 0 or 2,500 mg/kg of diet for 28 days. At the end of the feeding regimen, rats were either killed or maintained on control diets and sacrificed 1, 4, or 8 weeks after termination of dosing. None of the permethrin-treated rats died during the dosing period, but food consumption and body weights were reduced. In contrast, the animals gained weight rapidly after the dosing period, and no differences in body weight between control and permethrin-treated animals were observed at the end of the study period. After 4 weeks of permethrin dosing, significantly higher absolute and relative liver weights were observed. During the 8-week recovery period, relative liver weights of permethrin-treated animals were significantly higher than liver weights of control animals, but absolute liver weights of control and test animals were similar. Oxidative enzyme activity in liver microsomes was significantly higher in permethrin-treated animals than in controls at the end of dosing and 1 week later. The activity of liver microsomal enzymes was normal 4 weeks after dosing in the permethrin-treated animals. The amount of smooth endoplasmic reticulum in rat liver cells was significantly increased as a result of permethrin dosing, but within 4 weeks after dosing, no significant histological differences were observed in the livers of treated and control animals.

CD rats (six of each sex per group) permethrin at 0, 30, 100, 300, 1,000, or 3,000 mg/kg of diet for 5 weeks ([redacted]). Persistent tremors were seen in animals fed at 3,000 mg/kg, but none died. Growth was inhibited at that dose in both male and female rats. Relative liver weights were increased in male rats (groups fed 1,000 mg/kg of diet or higher) and female rats (fed 3,000 mg/kg). Histopathological examination of tissues and organs of the animals receiving the two highest doses did not show any adverse effects due to permethrin ingestion in the diet.

Sprague-Dawley rats (10 of each sex per group) were fed permethrin in the diet for 90 days at 0, 9, 27, 85, 270, or 850 mg/kg per day ([redacted]). All rats surviving to term were killed, and various tissues and organs from each animal were examined histopathologically. All male and female rats in the 850-mg/kg group died. An increase in the average liver-to-body-weight ratio was noted in both male and female rats fed 270 mg/kg. Compound-related histological changes were not observed in any of the tissues and organs examined. The minimum-effect dose was 270 mg/kg per day. At 85 mg/kg, no effects were observed.

Long-Evans rats (10 of each sex per group) were fed permethrin in the diet at 0, 20, 100, or 500 mg/kg of diet for 90 days ([redacted] . None died, and growth and food consumption of all animals were normal. The results of haematology, clinical chemistry, urinalysis, and ophthalmological examinations were also normal. Tremors were observed in

some animals at the highest dose, mainly during the first week of treatment. Significant increases in absolute and relative liver weights were observed at the two highest doses. Those increases were consistent with data from microscopic examination of the liver showing compound-related centrilobular hepatocyte hypertrophy in both males and females. There were no significant effects at the 20 mg/kg dose, although slight hepatic effects were reported in a few of the male rats.

Male Wistar rats were administrated permethrin (75 mg/kg/day, PO; 1/20 LD_{50}) daily for consecutive 90 days ([redacted]). The urine samples from Days 30, 60, and 90 after the first dosing were collected and analysed by ^{1}H NMR spectrometry. Serum biochemical analysis was also carried out. Permethrin caused significant changes in the urine metabolites such as taurine, creatinine, acetate, lactate, dimethylamine, dimethylglycine, and trimethylamine-N-oxide. These biological markers indicated prominent kidney and liver toxicity induced by permethrin. However, there was no change in serum biochemical parameters for the toxicity, indicating that metabolomic approach was much more sensitive in detecting the chronic toxicity.

Sprague-Dawley rats (16 of each sex per group) were fed permethrin in their diet at 0, 375, 750, 1,500, or 3,000 mg/kg of diet for 6 months ([redacted]). None died, and all animals exhibited normal growth and normal food and water consumption. Urinalysis and haematological and clinical biochemistry values were within normal limits. Signs of hyperexcitability and tremors were observed during the study in animals given 3,000 mg/kg, and their liver weights and liver-to-body-weight ratios were slightly increased. No significant histopathological findings were attributable to the presence of permethrin in the diet. The NOEL was 1,500 mg/kg.

Liver hypertrophy was evaluated in male and female Wistar rats that were fed permethrin at 0, 20, 100, or 1,000 mg/kg of diet for 26 weeks ([redacted] None died, and growth and food consumption were normal. Although the mean liver weight was increased at all doses, a significant increase was observed only at the highest dose. The increase in liver weight at that dose was accompanied by an increase in the smooth endoplasmic reticulum and in biochemical changes associated with microsomal oxidative mechanisms. In the 100 mg/kg group, there were slight, insignificant increases in biochemical activities. No effects on any of the values were observed in animals receiving 20 mg/kg.

Dog

Beagle dogs (four of each sex per group) were fed permethrin in gelatine capsules daily for 3 months at doses of 0, 5, 50, or 500 mg/kg ([redacted]). None died, but clinical signs of poisoning were observed at various times in both males and females at the highest dose. Food consumption and growth as well as clinical chemistry, haematological, and urinalysis values were normal. The liver weights and liver-to-body-weight ratios of animals

that received permethrin at 50 mg/kg or more were significantly increased. Histopathological examination did not show any adverse changes attributable to permethrin treatment.

Beagle dogs (four of each sex per group) were administered permethrin in gelatine capsules daily for 13 weeks at doses of 0, 10, 100, and 2,000 mg/kg ([redacted]). Permethrin treatment did not result in increased mortality, but clinical signs of poisoning were observed in the dogs in the 2,000-mg/kg group. Haematological, clinical chemistry and urinalysis values were within normal limits in all animals ([redacted]). There was a slight increase in the liver weight of animals receiving 2,000 mg/kg/day but there were no accompanying histopathological changes in the liver

Two Beagle dogs were administered daily oral doses of permethrin (*cis/trans* ratio, 25:75) at 500 mg/kg for 14 days ([redacted]). No clinical signs of toxicity or significant effects of the treatment on body weight or on clinical chemistry or haematological values were observed.

Beagle dogs (four males and four females in each group) were administered encapsulated permethrin (*cis/trans* ratio, 25:75) at doses of 0, 10, 50, or 250 mg/kg for 6 months ([redacted]). No signs of toxicity and no effect on body weight were seen. No gross pathological or significant histopathological findings were seen. Haematological and clinical chemistry values were within normal limits.

Rabbit

Five female Dutch rabbits were administered permethrin by gavage in 10 daily doses in corn oil at 0, 200, 400, or 800 mg/kg ([redacted]). The animals were sacrificed on Day 11. One rabbit, receiving 400 mg/kg exhibited mild hyperactivity and muscular fasciculation, but only on Days 6 and 7. Although all animals, including the controls, exhibited some degree of weight loss, it was most marked in the high dose group. There were no significant haematological or clinical chemistry findings.

Dermal Exposure

Technical-grade permethrin was applied daily to the clipped skin of New Zealand White rabbits (8 males / group) at dose levels of 0, 0.10, 0.32, or 1.0 mg/kg for 21 consecutive days (*IPCS, Permethrin, 1990*). The application site was abraded on the first test day in half (four) of the animals in each group. Blood samples were drawn weekly from the animals for clinical chemistry studies. All animals were killed on Day 10 after permethrin treatment was terminated. Various tissues and organs were removed from each animal and examined for microscopic lesions. A moderate primary irritation of the skin was produced by permethrin. No significant changes in body weight, organ weight, or clinical chemistry values were observed. No compound-related lesions in the skin or other tissues were observed.

Permethrin (dissolved in acetone) or acetone (as a control) was also applied on the skin twice a week for 3 weeks to six groups of 10 shaved male New Zealand White rabbits ([redacted]). Cotton cloth treated with permethrin (0.125 or 1.25 mg/cm2) was applied

to the skin over 1 mL of artificial sweat. The solution contained lactic acid, sodium chloride, urea, potassium chloride, glycine, glucose, ammonium hydroxide, and distilled water. In the case of other rabbits similarly treated, the sweat was omitted. In the control groups, acetone-treated cotton cloth with or without 1 mL of sweat was used. Blood samples were collected once a week for clinical chemistry determinations. All animals surviving to term were killed, and various tissues and organs from each animal were examined. No significant changes were noted in rabbit body weight or organ-to-body-weight ratios at the end of the 21- day test, and no skin irritation was observed. There were no significant changes in clinical chemistry values in the treated groups and no compound-related lesions on the skin or in other tissues and organs examined. The available information on subacute exposures shows that subacute exposure to permethrin is unlikely to cause dermal effects.

Inhalation Exposure

Inhalation toxicity of technical-grade permethrin was evaluated in Sprague-Dawley rats, guinea pigs, and Beagle dogs ([redacted] . The animals were exposed to an aerosol of permethrin at concentrations of 125, 250, or 500 mg/m^3, 6 hr per day, 5 days per week for 13 weeks. At 500 mg/m^3, tremors and convulsions were observed in the rats during the first week of exposure but disappeared in the second week. Urine metabolite studies indicated that permethrin was rapidly metabolized and excreted. Post-exposure experiments in male rats showed that the hexobarbital-induced sleeping time was significantly shortened after exposures at 500 mg/m^3 but not at lower doses. No clinical signs of permethrin toxicity were observed in the guinea pigs and dogs when exposed to aerosols of permethrin under similar conditions. Pulmonary function, clinical chemistry values, and blood-cell counts were normal. No compound-related gross or microscopic pathological changes were observed in the dogs, rats, or guinea pigs due to permethrin inhalation.

Oxidative Stress

It has been suggested that permethrin might exert a variety of toxic effects on animals and humans alike, such as neurotoxicity, immunotoxicity, cardiotoxicity, hepatotoxicity, reproductive, genotoxic, and haematotoxic effects, digestive system toxicity, and cytotoxicity via oxidative stress ([redacted] .

2.4.4.3 Genotoxicity

Permethrin have been tested for its ability to produce mutations in Ames reverse mutation assay using *Salmonella typhimurium* tester strains TA 1535, TA 1537, TA 1538, TA 98, and TA 100, with and without metabolic activation ([redacted]). The concentrations of permethrin tested ranged from 1 to 2,500 µg per plate. The results showed that permethrin was not mutagenic in the Ames *Salmonella* test. Permethrin was also not mutagenic in *Escherichia coli* WP_2 uvrA mutation assay. In one host-mediated assay, permethrin (200 mg/kg) was orally administered to ICR mice, and the indicator organisms, *S. typhimurium* G46, were injected IP and harvested from the abdominal cavity of mice 3 hr after treatment. The study did not reveal

any mutagenic effect. In another host-mediated assay employing a similar test system, *trans*-permethrin at 600 and 3,000 mg/kg and *cis*-permethrin at 21 and 54 mg/kg gave negative results ([redacted]). Permethrin has also been tested for its genotoxicity with *Saccharomyces cerevisiae* D4, with or without metabolic activation, at concentrations ranging from 0.0001 to 5.0 µg per plate, and the results showed that permethrin was not mutagenic to *S. cerevisiae* D4. Permethrin was not mutagenic in *Drosophila melanogaster* sex-linked recessive lethal mutation assay ([redacted] , and to V79 Chinese hamster cells, with or without metabolic activation ([redacted]).

The results of investigations of clastogenic effects of permethrin are inconsistent. In one study, *D. melanogaster* males were administered permethrin in a feeding solution at 5 ppm for 3 days before mating with untreated mus-302 DNA-repair defective females ([redacted]). The F1 male progeny were screened for partial or complete chromosomal loss. The results of this investigation were negative.

Permethrin was tested for its ability to induce micronuclei in Alderley Park rats ([redacted] . Male rats (12 controls and 8 per treatment group) were injected IP with permethrin (94% pure, *cis*/*trans* ratio, 40:60) once or in five daily doses of 0, 600, 3,000, or 6,000 mg/kg. There were 8 animals per test group and 12 animals in the control group. Rats were killed 24 h after the single injection and 6 h after the final dose with multiple injections. Bone-marrow cells from each animal were analysed, and the results of this investigation showed that permethrin was not clastogenic.

Permethrin was tested for its ability to induce sister chromatid exchanges (SCEs), micronuclei, and chromosomal aberrations in cultured human peripheral blood lymphocytes from two human donors [redacted]. Permethrin was applied at concentrations of 5-200 µg/mL in the absence and presence of rat liver S9 mix. Small increases in the SCE frequencies were found that were statistically significant, but they might not be biologically meaningful since there was no dose-effect relationship and the increase in SCEs was not always reproducible. Permethrin increased the occurrence of micronuclei over controls when it was assayed at concentrations of 10-100 µg/mL in the absence of S9 mix. The effect was statistically significant. However, in the presence of S9 mix, the increase in micronuclei was not statistically significant in lymphocytes from both human donors. Permethrin was found to increase the frequency of chromosomal aberrations at concentrations of 75-150 µg/mL in the absence of S9 mix. In the presence of S9 mix, the increase in frequency of chromosomal aberrations was not statistically significant.

The induction of SCEs was studied in cultured human lymphocytes from two human donors ([redacted]). Permethrin induced SCEs in the absence of rat liver S9 mix at concentrations of 50 or 100 µg/mL; however, the increases in frequency of SCEs were not dose-related. There were differences between the two donors in the SCE assays carried out in the presence of liver S9 mix - one showed no increase in SCEs and the other showed an increase

in SCEs that was not dose-related. The induction of micronuclei was also studied in cultured human lymphocytes. The lymphocytes were exposed to permethrin at concentrations of 0, 10, 25, or 50 µg/mL in the absence of S9 mix. Permethrin was positive in the micronucleus test when it was assayed in the absence of S9 mix. However, in the presence of S9 mix and permethrin concentrations of 0, 25, 50, 75, 100, or 200 µg/mL, the increase in number of micronuclei, observed in some cases, was not statistically significant.

In DNA damage studies, permethrin was negative in the *E. coli* pol A assay, the *Bacilus subtilis* rec assay, the *S. cerevisiae* D3 mitotic recombination assay, and the unscheduled DNA synthesis in human lung fibroblasts ([redacted] .

2.4.4.4 Carcinogenicity

Mice

A chronic carcinogenicity study was conducted in 70 Alderley Park (Swiss-derived) mice of each sex per group (*NCR US, 1994*). The animals were administered permethrin (purity, 94.0-98.9%; *cis*/*trans* ratio, 40:60) at 0, 250, 1,000, or 2,500 ppm (0, 37.5, 150, or 375 mg/kg per day) for 98 weeks. A slight increase was reported in lung adenomas in males at the highest dose tested (2,500 ppm), which was not statistically significant. There were no carcinomas observed in any of the male groups; in female mice, one carcinoma was reported in each treatment group. There was a slight decrease in growth of animals administered the two highest doses of permethrin. Nononcogenic effects noted at doses of 1,000 ppm and above included minimal changes in liver enzyme activity, increases in liver weight, and histopathological changes in the liver (eosinophilia of hepatocytes). Hepatic aminopyrine N-demethylase activity increased significantly in male and female mice in the highest-dose group. A decrease in vacuolation of the proximal tubular epithelium of the kidney was also noted at all doses of permethrin in male mice. At the highest dose tested (2,500 ppm), mortality increased in both sexes. The NOEL for this study was 250 ppm (37.5 mg/kg per day) based on the liver effects.

Permethrin (purity, 94.5-96.7%; *cis*/*trans* ratio, 40:60) was administered to groups of 75 male and 75 female Charles River CD-1 mice for 2 years ([redacted]. Male mice were fed permethrin at 0, 20, 500, or 2,000 ppm in diet (equivalent to 0, 3, 71, or 286 mg/kg per day); female mice were fed permethrin at 0, 20, 2,500, or 5,000 ppm (0, 3, 357, or 714 mg/kg per day). Survival decreased at high doses in male mice at both mid and high doses. Non-oncogenic effects observed during the study consisted of increased mortality in males at 2000 mg/kg diet, increased liver weight in females at 2500 mg/kg and 5000 mg/kg, and increased lung weight in females at 5000 mg/kg. Histopathologically, dose-related “focal alveolar cell proliferation" (increased numbers of lung cells) was observed in permethrin-treated females. Concerning oncogenic effects, there was an increased incidence of bronchioalveolar adenomas in female mice only. The number of female mice with adenomas and/or carcinomas (15/74, 24/72, 35/74, and 44/75 at the 4 dose levels) revealed a statistically significant dose-response relationship. Male mice did not show this effect. The Federal Insecticide, Fungicide, and

Rodenticide Act (FIFRA) Scientific Advisory Panel expressed some doubt concerning the conduct of this study.

Permethrin (*cis/trans* ratio, 25:75) was administered to groups of 75 male and 75 female (100 mice of each sex for control) CFLP-strain Swiss-derived mice at 0, 10, 50, or 250 mg/kg/day for 92 weeks ([redacted]). There were statistically significant increases in benign lung tumours at the highest dose in females and a significant dose-related trend. Malignant lung tumours were observed in treated animals (one in the mid- and two in the high dose group), and none were seen in controls. The tumour incidences were, however, within the historical range. Non-oncogenic effects observed during the study consisted of slightly decreased mortality in females at 50 and 250 mg/kg/day, increased liver weights in males, and increased kidney weights in females at 250 mg/kg/day. Histopathologically, an increased incidence in cuboidal/columnar metaplasia of the alveolar epithelium was observed in the lungs of male and female mice at the highest dose. The oncogenicity data indicated a dose-related trend in females, but not in males, for adenomatous tumours in the lungs.

A 24-month oral carcinogenicity study of permethrin was conducted by feeding male and female CD-1 mice diets containing concentrations of 0, 20, 500, and 2,000 ppm of permethrin (males) or 0, 20, 2,500, and 5,000 ppm of permethrin (females) ([redacted]). Chronic dietary exposures to permethrin induced increased incidences of hepatocellular adenoma in female mice at concentration levels of 2,500 ppm or higher, when compared to controls. Permethrin exposure did not increase the incidence of hepatocellular carcinoma in either male or female mice.

Rats

Permethrin has been tested for its carcinogenicity in rats. Wistar rats (60 of each sex per group) were fed permethrin (purity 93.1-98.9%; *cis/trans* ratio, 40:60) at 0, 500, 1,000, or 2,500 ppm (0, 25, 50, or 125 mg/kg/day) for 2 years. No carcinogenic effects were noted at any concentration ([redacted] . Signs of poisoning, such as tremors and hyperexcitability, were noted during the first 2 weeks of dosing in the animals that received the highest dose. There was no mortality attributable to permethrin, and growth and food consumption were unaffected. There were no effects on haematological, ophthalmological, urological, or other clinical chemistry measurements. Liver aminopyrine N-demethylase activity was increased in all permethrin-treated animals. Bone-marrow smears of the animals showed no unusual findings. Gross and microscopic examination of tissues and organs was performed after 1 and 2 years, and all animals that died with neoplastic changes were examined. Kidney weight was also increased, predominantly in males, at all doses. Examination of the sciatic nerve showed no effects attributable to permethrin. Liver weights were higher in male and female rats that received permethrin for 1 year at 2,500 ppm (25 mg/kg) than in the control animals. After 2 years, the liver weight and liver-to-body-weight ratios were higher in all permethrin-treated

males than in the corresponding controls. In the females, higher values of absolute and relative liver weights, compared with the controls, were recorded only in the group of animals given 1,000 mg/kg. Hepatocyte vacuolation was seen at 1 year in males fed at only the highest dose and in females at all doses. The smooth endoplasmic reticulum showed significant increases at 52 weeks in males and females at all doses. At the end of the study, notable endoplasmic reticulum increases were detected only at the highest doses, although insignificant increases were noted at all doses in males and females. The NOEL for liver effects was 500 ppm (25 mg/kg/day) and the lowest-observed-effect-level (LOEL) was 1,000 ppm (50 mg/kg/day).

Long-Evans rats (60 males and 60 females per group) were fed permethrin (purity unknown; *cis*/*trans* ratio, 40:60) at 0, 20, 100, or 500 ppm (0, 1, 5, 25mg/kg per day) for 2 years (*NCR US, 1994*). Two independent evaluations of the histopathological data from this study concluded that there was no carcinogenic potential for permethrin. Although there was a dose dependent increase in gross liver weight in both males and females, those values were small and not statistically significant. The initial examination of lung tissue from males suggested that there was a dose-related increase in lung tumours, although the difference was not statistically significant at any dose, nor was there a significant dose-related trend. The major deficiency in the study was that there was no clear evidence of toxicity even at the highest dose tested. There was no mortality, and the animals did not reveal adverse effects on growth, food consumption, or behaviour attributable to the administration. Haematological, clinical chemistry and urinalysis measurements were performed at either 6 months or 1 year and at the end of the study. There were no compound-related effects on a wide variety of parameters examined, and ophthalmological examination indicated no abnormalities.

Seventy-five Wistar rats of each sex per group were given permethrin (purity unknown; *cis*/*trans* ratio, 25:75) in the diet at 0, 10, 50, or 250 mg/kg per day for 103 weeks ([redacted]. No evidence of carcinogenicity of permethrin was reported in the study. Hepatocyte hypertrophy was observed histopathologically in both sexes at mid and high doses. The NOEL and LOEL were 10 and 50 mg/kg/day, respectively, based on liver hypertrophy.

2.4.4.5 Reproductive and Developmental Toxicity

ICR mice were treated with 0, 15, 50, or 150 mg/kg, PO on gestation days (GDs) 7-12 ([redacted] Two-thirds of the animals were killed on GD 18, and the rest went to term, delivered, and weaned their young; pups were killed at 6 weeks of age. No effects were seen on any maternal, developmental, or postnatal endpoints.

Groups of Long-Evans rats (10 males and 20 females per group) were fed permethrin at dose levels of 0, 20, and 100 mg/kg diet in a 3-generation (two litters per generation) reproduction study ([redacted]. There was no effect on mortality, mating, pregnancy, or fertility, with the exception of the F2 mating index, which was reduced in both controls and treatment groups. Pup survival and growth were unaffected. Haematological evaluations of F2

adults between the second and third mating showed no unusual effects. This study indicated that dietary permethrin does not adversely affect reproduction in the rat.

Groups of Wistar rats (12 males and 24 females per group) were fed permethrin at dose levels of 0, 500, 1000, and 2500 mg/kg diet for 12 weeks ([redacted]). At 12 weeks, the animals were mated to initiate a standard 3-generation (two litters per generation) reproduction study. Clinical signs of acute poisoning (tremors, etc.) were noted, predominantly in females given the highest dose. There were no effects attributable to permethrin on fertility, gestation, viability of pups, sex ratio, litter size, or lactation. Ten male and female weanlings from the second litter of the F3 generation were examined for histopathological changes. Centrilobular hypertrophy and cytoplasmic eosinophilia were observed at all dose levels, the incidence and severity of which appeared to be dose dependent. Rats in the third litter of the F3 generation were sacrificed on Day 12 of gestation for teratogenic examination, but no abnormalities were observed. Based on the results of this study, permethrin does not appear to induce reproductive toxicity in rats.

Female Sprague-Dawley rats (5-8 rats per group) were fed permethrin in the diet at levels of 0, 500, 1000, 1500, 2000, 2500, 3000, 3500, and 4000 mg/kg diet from day 6 to day 15 of pregnancy ([redacted]). Laparotomy was performed on Day 20 of gestation, and the number of live foetuses was determined. Placentae were excised and cleaned of extraneous connective tissue. Analysis of the protein and glycogen contents of the placentae on Day 16 of pregnancy indicated that they were only influenced by very high doses (2500-4000 mg/kg diet) of permethrin. Analysis of variance indicated no significant effect on protein level, but the treatment did decrease the glycogen concentration. No significant dose-related effects on implantational sites/intrauterine foetuses were observed. These results appeared to confirm that permethrin possesses low mammalian toxicity.

In a 3-generation reproduction study, groups of 20 male and 20 female Wistar COBS rats received permethrin (25:75) in the diet at 0, 5, 30, and 180 mg/kg per day during growth, mating, gestation, parturition, and lactation for three generations, each with two litters ([redacted]). Foetal toxicity and teratogenicity was assessed in the second pregnancy of the F2 generation. Treatment with permethrin had no effect on general behaviour or condition, food intake, body weight gain, or pregnancy rate of the dams, or on parturition, sex ratio, or pup weight. A small number of rats of each group developed eye abnormalities, including ocular haemorrhage and chronic glaucoma, but this was not related to the treatment. Examination of F3b foetuses showed no treatment-related effect on sex ratio, body weight, or the occurrence of visceral or skeletal abnormalities. This study indicated that permethrin (25:75) has no effect on the reproduction of rats at doses up to 180 mg/kg per day.

Dutch rabbits were administered permethrin by gavage at 0, 600, 1,200, or 1,800 mg/kg in 0.5% Tween 80 on GDs 6-18 and killed the animals on GD 29 ([redacted]). Maternal body-weight gain was reduced, and there was increased hair loss at 1,200 and 1,800 mg/kg. Embryo

lethality was also increased at the two highest doses, and foetal weight was decreased at 1,800 mg/kg.

2.4.4.6 Other Toxicity Studies

The most significant (morphologically recognizable) toxicological effect of permethrin involves the liver. It is characterized by an increase (absolute and relative) in weight, but takes several repeated exposures to be evident ([redacted]. The lowest dose that has been reported to cause a significant increase in liver weight occurred after animals ingested 100 mg/kg for 26 weeks. Sex-related differences in liver enlargement have been reported but are not consistent. Female rats exhibited an increase in liver weight and in liver-to-body-weight ratio when fed a diet containing permethrin at 1,000 mg/kg of diet, whereas that effect was observed in male rats at 2,500 mg/kg of diet. The increase in liver weight is due to hepatocellular hypertrophy. The NOEL for hepatocellular hypertrophy in rats has been estimated to be 10 mg/kg/day based on a LOEL of 50 mg/kg after 2 years of oral exposure (in feed); the NOEL for hepatic enzyme induction was estimated to be 25 mg/kg and the LOEL to be 50 mg/kg ([redacted]

2.4.4.7 Local Tolerance

In an acute dermal toxicity test in rats, LD_{50} was greater than 200 mg/kg, and desquamation, oedema, thickening, scab, or skin eruptions were observed in 9 of 10 rats ([redacted]). In the rabbit, skin irritation responses were evaluated to several concentrations of permethrin. At a concentration of approximately 80 mg/cm^2, erythema and oedema were observed ([redacted], [redacted]. When a permethrin formulation was applied to the clipped dorsal surface (0.13 mg/cm^2) of six New Zealand White rabbits (three of each sex) once a day for 16 days, a slight erythema appeared which correlated with increased cutaneous blood flow. No significant histopathological changes were detected ([redacted]).

In a dermal sensitization, guinea pigs were dermally administered permethrin as a 10% solution in dimethylformamide for 3 consecutive days. This was followed 4 days later by challenge doses of 0.1%, 1%, and 10% solutions of permethrin in dimethylformamide. Only very slight erythema was observed. Permethrin was therefore considered either non-sensitizing or only mildly so ([redacted]. Irradiation of permethrin-pretreated guinea pigs with ultraviolet (UV) light (365 nm) for 30 min at a distance of 10-15 cm did not cause a photochemical irritation reaction.

The ocular irritation of permethrin has been tested in the eyes of Japanese White rabbits. No eye irritation was observed in the rabbits ([redacted]).

2.4.4.8 Ecological Toxicity

The environmental fate of permethrin is strongly influenced by its extreme hydrophobicity, low solubility, and high tendency to adsorb to soil ([redacted]).

Permethrin is largely associated with sediment in aquatic systems and immobile in soil making it unlikely to reach to ground water. Microbial degradation and photolysis are important degradation routes while volatilization and hydrolysis are relatively unimportant dissipation routes. Permethrin has a relatively low mammalian and avian toxicity, but is potentially very toxic to aquatic organisms, especially invertebrates. The highest risk for aquatic toxicity in the water column is immediately after application. After application to water, permethrin binds quickly to sediment due to its hydrophobic behaviour, and becomes less bioavailable to organisms. While permethrin does have a tendency to bioconcentrate, aquatic organisms have demonstrated the ability to depurate permethrin through excretion in some studies.

2.4.4.9 Toxicology of Components of the Formulation

The proposed product Permethrin Dermal Cream 5% w/w is a generic version of Lyclear Dermal (Permethrin 5%) Cream, the MA holder of which is Omega Pharma Ltd.. The Lyclear Dermal (Permethrin 5%) Cream is indicated for the treatment of scabies in adults and children and crab lice in adults, and is marketed in the EU, USA and in a number of non-EU countries for more than 10 years.

The concentration of each excipient was optimized based on the reverse engineering/deformulation of reference product for glycerol, macrogol (2) cetyl ether, isopropyl myristate, butylated hydroxytoluene and purified water. Different trials were taken during development to optimize the concentration of the remaining excipients. The concentration of each excipient was compared with the Inactive Ingredient Database (IID) of the Food and Drug Administration (FDA) (https://www.accessdata.fda.gov/scripts/cder/iig/index.cfm). The IID provides information on inactive ingredients present in approved drug products. For new drug development purposes, once an inactive ingredient has appeared in an approved drug product for a particular route of administration, the inactive ingredient is not considered new and may require a less extensive review the next time it is included in a new drug product. If a particular inactive ingredient has been approved in a certain dosage form at a certain potency, it may be considered safe for use in a similar manner for a similar type of product. Table 5 lists the excipients used in the proposed product Permethrin Dermal Cream 5% w/w, their monograph status, the % w/w concentration, and reference to topical products as per IID containing ≥ concentration of a particular excipient compared to that in the Permethrin Dermal Cream 5% w/w.

Table 5. List of excipients used in the proposed product Permethrin Dermal Cream 5% w/w, their monograph status, % w/w concentration, and reference to topical products as per IID

Excipients	Monograph status	% w/w	As per IID	
			Dosage form	Max potency per unit dose (% w/w)
Butylated Hydroxy Toluene	Ph Eur.	[redacted]	augmented cream	0.2
Carbomer	Ph Eur.	[redacted]	gel	1.51
Coconut Oil, Refined	Ph Eur.	[redacted]	ointment	20.0
Glycerol	Ph Eur.	[redacted]	emulsion cream	20
Glycerol Monostearate 40-55	Ph Eur.	[redacted]	emulsion, cream	20
Isopropyl Myristate	Ph Eur.	[redacted]	emulsion cream	15
Liquid paraffin mixture	IH*	[redacted]	Ointment	5
Macrogol (2) Cetyl ether	IH	[redacted]	emulsion cream	2.5
Methyl Parahydroxy Benzoate	Ph Eur.	[redacted]	emulsion cream	0.5
Propyl Parahydroxy Benzoate	Ph Eur.	[redacted]	emulsion cream	5.25
Sodium Hydroxide	Ph Eur.	[redacted]	cream	2.72

*IH – In-house

As demonstrated by Table 5, the concentration of each excipient used in the formulation of Permethrin cream 5% was less than that of the Reference Listed Drug (RLD) with the exception of Macrogol (2) cetyl ether, the concentration of which is the same as the RLD. Detailed description of excipients is as follows:

Butylated hydroxytoluene (BHT), also known as dibutylhydroxytoluene, is a lipophilic organic compound, chemically a derivative of phenol that is useful for its antioxidant properties. BHT is used as an antioxidant in a wide range of cosmetic formulations at concentrations from 0.0002% to 0.5% ([redacted]). European and US regulations allow small amounts to be used as a food additive. BHT does penetrate the skin, but the relatively low amo unt absorbed remains primarily in the skin. BHT applied to the skin was associated with toxic effects in lung tissue. BHT was not a reproductive or developmental toxin in animals. BHT has been found to enhance and to inhibit the humoral immune response in animals. BHT itself was not generally considered genotoxic, although it did modify the genotoxicity of other agents. BHT has been associated with hepatocellular and pulmonary adenomas in animals, but was not considered carcinogenic and actually was associated with a decreased incidence of neoplasms. In a predictive clinical test, 100% BHT was a mild irritant and a moderate sensitizer. In provocative skin tests, BHT (in the 1% to 2% concentration range) produced positive reactions in a small number of patients. Clinical testing did not find any depigmentation associated with dermal exposure to BHT, although a few case reports of depigmentation were found. BHT applied to the skin appears to remain in the skin or pass through only slowly and does not

produce systemic exposures to BHT or its metabolites seen with oral exposures. Although there are only limited studies that evaluated the effect of BHT on the skin, the available studies, along with the case literature, demonstrate no significant irritation, sensitization, or photosensitization. Recognizing the low concentration, at which this ingredient is currently used in topical formulations, it was concluded that BHT could be safely used ( ).

Carbomer 974P - Poly(acrylic acid) (PAA or Carbomer) is generic name for synthetic high molecular weight polymers of acrylic acid. PAA and its derivatives are used in disposable diapers, ion exchange resins and adhesives. They are also used as thickening, dispersing, suspending and emulsifying agents in pharmaceuticals, cosmetics and paints. Rat LD_{50} dose: 2500.00 mg/kg, PO. Mouse LD_{50} dose: 4600.00 mg/kg, PO. Chronic oral toxicity: No significant effect was observed in rats or dogs fed with resin as 5% of diet for 6-1/2 months (. The safety and efficacy of 0.2% PAA gel and polyvinylalcohol 1.4% (PVA) was compered in the treatment of patients with dry eyes ( ). Eighty-nine patients with dry eyes were randomly allocated to treatment with either PAA (48) or PVA (41) in a prospective, investigator-masked study in two centres. Both PAA and PVA were safe and equally well-tolerated except for blurred vision, usually mild and transient, on PAA. On global assessment of the improvement in their dry eye condition, polyacrylic acid gel was as safe as and more effective than polyvinylalcohol in the treatment of patients with dry eyes.

Coconut oil, Refined - Coconut oil, or copra oil, is an edible oil extracted from the kernel or meat of mature coconuts harvested from the coconut palm (*Cocos nucifera*). It has various applications. Refined coconut oil is used for home cooking, commercial food processing, and cosmetic, industrial, and pharmaceutical purposes. Safety assessment by animal toxicology studies concluded that coconut oil and hydrogenated coconut oil are relatively nontoxic by ingestion and that hydrogenated coconut oil was nontoxic, nonirritating, and not a sensitizer (. In an Ames test conducted without metabolic activation, saponified coconut oil (SCO) was not mutagenic for *Salmonella typhimurium* strain TA98, but it displayed mutagenic potential for strains TA100 and TA104. The cytotoxic, antioxidant, and mutagenic effects of SCO can be influenced by the aggregational state. Coconut oil was not an allergen at 100% concentration in 12 participants in a double-blind randomized controlled pilot study ( ).

Glycerol, E422 is a humectant and sweetener. Glycerine is classified by the FDA as "generally recognized as safe" (GRAS) and complies with specifications for the Food Chemicals Codex (FCC), United States Pharmacopeia (USP), and European Pharmacopoeia (Ph.Eur. or EP) ( ). The Panels on Food Additives and Nutrient Sources Added to Food (ANS) provides a scientific opinion re-evaluating the safety of glycerol (E 422) used as a food additive (. Glycerol has low acute toxicity, and did not raise

concern with respect to genotoxicity and carcinogenicity. Reproductive and prenatal developmental studies were limited to conclude on reproductive toxicity but no dose-related adverse effects were reported. None of the animal studies available identified an adverse effect for glycerol. The Panel conservatively estimated the lowest oral dose of glycerol required for therapeutic effect to be 125 mg/kg/h and noted that infants and toddlers can be exposed to that dose by drinking less than the volume of one can (330 mL) of a flavoured drink.

Glycerol monostearate 40-55 - Glycerol monostearate, commonly known as GMS, is an organic molecule used as an emulsifier. GMS is a white, odourless, and sweet-tasting flaky powder that is hygroscopic. It is a glycerol ester of stearic acid. It occurs naturally in the body as a by-product of the breakdown of fats, and is found in fatty foods. GMS is a food additive used as a thickening, emulsifying, anti-caking, and preservative agent; an emulsifying agent for oils, waxes, and solvents; a protective coating for hygroscopic powders; a solidifier and control release agent in pharmaceuticals; and a resin lubricant ([redacted], [redacted]. GMS is used in cosmetic formulations at concentrations of ≥ 0.1-50%. In acute oral toxicity studies in rats, GMS was nontoxic or mildly toxic (*ACT, Glyceryl stearate SE, 1982*). In chronic studies, 15-25% GMS in the diet of rats for three consecutive generations had no adverse effects. Rats fed a diet containing 25% GMS for two years developed renal calcifications. GMS at concentrations of up to 100% were reported to be mildly irritating or non-irritating to the skin of rabbits. In subchronic and chronic dermal toxicity tests, 4-5% GMS was non-toxic to rabbits but did cause moderate irritation (slight to moderate erythema, oedema, atonia, desquamation, and/or fissuring). In seven guinea pig sensitization studies, GMS did not induce sensitization. In primary eye irritation studies, GMS at concentrations up to 100% were mildly irritating or non-irritating when instilled in the eyes of rabbits. GMS fed to mice in doses of 50-100 mg/day or 1.5% in the diet until they died did not induce significant brain or gastric tumour formation, respectively. Five percent GMS did not promote the carcinogenicity of 7,12-dimethylbenz[a]anthracene (DMBA) in mouse skin. Single and Repeated Insult Patch Tests used to evaluate human skin irritation and sensitization potential of GMS showed GMS to be non-sensitizing and non-irritating. Products containing 2% GMS were non-phototoxic and non-photoallergic. Worker experience shows that GMS is non-irritating to human skin.

Isopropyl myristate is the ester of isopropanol and myristic acid. Isopropyl myristate is used in cosmetic and topical medicinal preparations where good absorption through the skin is desired. It is also used as a pesticide-free treatment against head lice, which works by dissolving the wax that covers the exoskeleton of head lice, killing them by dehydration. Hazardous in case of ingestion. Slightly hazardous in case of skin contact (irritant, permeator), of inhalation ([redacted]). In a repeated patch-test 1.28-1.70 g isopropyl myristate was applied for 24 hr every other day for 15 applications to 50 health volunteers. The study did not detect any sensitizing potential ([redacted]). A bath oil containing 42.9% isopropyl myristate was tested for photocontact allergenicity on seven men

and 18 women. No evidence of photocontact allergenicity was observed. A phototoxicity study with a bath oil formulation containing 42.9% isopropyl myristate was conducted on one man and nine women. No evidence of phototoxicity was observed. Four separate human sensitization studies testing an aerosol antiperspirant concentrate containing 52-58% isopropyl myristate produced no reactions indicative of sensitization in a total of 320 subjects. A maximization test was carried out on 25 volunteers using 20% isopropyl myristate in petrolatum. A challenge patch with sodium lauryl sulphate pretreatment was made after a 10-day rest. No sensitization reactions were produced.

Liquid paraffin mixture - Medicinal liquid paraffin, also known as paraffinum liquidum, is a very highly refined mineral oil used in cosmetics and for medical purposes. This is a UK definition (British Pharmacopoeia) and the term may have different uses in other countries ([redacted]. The clinical efficacy and safety of polyethylene glycol (PEG) 3350 solution and liquid paraffin were evaluated in the treatment of children with functional constipation in Sari Toba clinic in Iran during the period of 2008-2009 ([redacted]. One hundred and sixty children 2-12 years old with functional constipation were included in this study. Patients received either 1.0-1.5 g/kg/day PEG 3350 or 1.0-1.5 ml/kg/day liquid paraffin for 4 months. Adverse reactions observed were nausea, vomiting, flatulence, abdominal pain and dehydration, diarrhoea. No serious or significant adverse events were observed. Based on animal studies and reports of negligible epidermal penetration of topically applied white mineral oils, there is no evidence of any hazard identified for topical exposure to white mineral oils at any dose in multiple species ([redacted]).

Macrogol (2) cetyl ether - Macrogol cetostearyl ether is a mixture of ethers of mixed macrogols with linear fatty alcohols, mainly cetostearyl alcohol. It may contain some free macrogols and contains various amounts of free cetostearyl alcohol. The amount of ethylene oxide reacted with cetostearyl alcohol is from two to thirty-three units per molecule (nominal value). For external application for use as an emollient, and for use as a diluent in medicinal external preparations. It is an emulsifying surfactant ([redacted]). There are no reports available on any toxic hazard of macrogol cetostearyl ether.

Methyl parahydroxybenzoate (E218), also methylparaben, is one of the parabens. Methylparaben is an anti-fungal agent often used in a variety of cosmetics and personal-care products. It is also used as a food preservative and has the E number E218. Its oral LD_{50} value in the mouse is >8000 mg/kg ([redacted]. Parabens are used in over 22,000 cosmetics as preservatives at concentrations up to 0.8% (mixtures of parabens) or up to 0.4% (single paraben) ([redacted]. The group includes methylparaben. Parabens do not accumulate in the body. Serum concentrations of parabens, even after IV administration, quickly decline and remain low. Acute toxicity studies in animals indicate that parabens are not significantly toxic by various routes of administration. Subchronic and chronic oral studies indicate that parabens are practically nontoxic. Numerous genotoxicity studies, including Ames

testing, dominant lethal assay, host-mediated assay, and cytogenic assays, indicate that parabens are generally non-mutagenic, although methylparaben did increase chromosomal aberrations in a Chinese Hamster ovary cell assay. Methylparaben was non-carcinogenic when injected SC in mice or rats or when administered intravaginally in rats, and was not cocarcinogenic when injected SC in mice. Methylparaben was non-teratogenic in rabbits, rats, mice, and hamsters. In one *in vitro* study, sperms were not viable at concentration as low as 6 mg/ml methylparaben, but an *in vivo* study of 0.1% or 1.0% methylparaben in the diet of mice reported no spermatotoxic effects. Methylparaben was studied using rats at levels in the diet up to an estimated mean dose of 1141.1 mg/kg/day with no adverse testicular effects. In skin irritation tests, methylparaben produced PIIs of 0.0 to 1.0 (out of 4.0), values indicative of no to mild irritation. Methylparaben (0.1%) injected intradermally into the shaved dorsal skin of four guinea pigs 5 days per week for 8 weeks had a desensitizing effect. According to a 2006 FDA report on the frequency of use of cosmetic ingredients, methylparaben was used in 8786 products across a wide range of product categories. Methylparaben is used to a total concentration of 0.3% in baby lotions, oils, powders, and creams. Methylparaben is used in baby cleansing cloths to a total concentration of 0.45%. The European Food Safety Authority (EFSA) Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food adopted an opinion on the safety of paraben usage in food in 2004, which stated that the ADI of 0 to 10 mg/kg/day for methylparaben is still valid ([redacted] .

Propyl parahydroxybenzoate (E216), propylparaben, the n-propyl ester of p-hydroxybenzoic acid, occurs as a natural substance found in many plants and some insects, although it is manufactured synthetically for use in cosmetics, pharmaceuticals and foods. It is a preservative typically found in many water-based cosmetics, such as creams, lotions, shampoos and bath products. As a food additive, it has the E number E216. Asthma-like symptoms may continue for months or even years after exposure to the material ceases. This may be due to a non-allergenic condition known as reactive airways dysfunction syndrome (RADS) which can occur following exposure to high levels of highly irritating compound. Studies showed increased mortality, reduced weight gain, liver and kidney effects at higher doses, also, lesions of the brains, thymus and skeletal muscles may occur with benzyl alcohol ([redacted]). Propylparaben was non-carcinogenic in a study of transplacental carcinogenesis [redacted]). Products containing 0.2% or 0.3% propylparaben caused no deaths when administered to rats at doses of 15 g/kg. In one *in vitro* study, sperms were not viabile at concentrations as low as 3 mg/ml propylparaben. Propylparaben did affect sperm counts at all levels from 0.01% to 1.0%. According to a 2006 FDA report on the frequency of use of cosmetic ingredients, methylparaben was used in 7118 products across a wide range of product categories. Propylparaben is used to a total concentration of 0.3% in baby lotions, oils, powders, and creams. Propylparaben is used in baby cleansing cloths to a total concentration of 0.45%. Propylparaben (21 CFR 184.1670) are

generally recognized as safe (GRAS) when used as chemical preservatives in foods, with use limits of 0.1% for each.

Sodium hydroxide (NaOH) is an inorganic compound. It is a white solid and highly caustic metallic base and alkali salt of sodium which is available in pellets, flakes, granules, and as prepared solutions at a number of different concentrations. Sodium hydroxide has many uses in cosmetics. Sodium hydroxide is also used as a technical ingredient, to adjust pH and to neutralise ingredients. Sodium hydroxide forms an approximately 50% (by weight) saturated solution with water. Lowest published lethal dose in rabbits is 500 mg/kg, PO [redacted]). Sodium hydroxide does not produce systemic toxicity, but is very corrosive and can cause severe burns in all tissues that it comes to contact with (*ATSDR, 2008*). Sodium hydroxide has not been classified for carcinogenic effects. Exposure to sodium hydroxide solid or solution can cause skin and eye irritation. Direct contact with the solid or with concentrated solutions causes thermal and chemical burns leading to deep-tissue injuries. Very strong solutions of sodium hydroxide can hydrolyze proteins in the eyes, leading to severe burns and eye damage or, in extreme cases, blindness. Inhalation of sodium hydroxide is immediately irritating to the respiratory tract. Children may be more vulnerable to corrosive agents than adults because of the relatively smaller diameter of their airways. Skin contact with solid sodium hydroxide or its concentrated solutions can cause severe burns with deep ulcerations. Burns appear soft and moist and are very painful. Although contact with concentrated solutions causes pain and irritation within 3 minutes, contact with dilute solutions may not cause symptoms for several hours. Because of their relatively larger surface area:body weight ratio, children are more vulnerable to toxicants affecting the skin.

Purified water, is water that has been mechanically filtered or processed to remove impurities and make it suitable for use. Distilled water has been the most common form of purified water, but, in recent years, water is more frequently purified by other processes including capacitive deionization, reverse osmosis, carbon filtering, microfiltration, ultrafiltration, UV oxidation, or electrodeionization. Combinations of a number of these processes have come into use to produce water of such high purity that its trace contaminants are measured in parts per billion (ppb) or parts per trillion (ppt). Purified water has many uses, largely in the production of medications, in science and engineering laboratories and industries, and is produced in a range of purities. Rat LD_{50} dose is > 90 ml/kg, PO. Non-corrosive for skin. Non-irritant for skin. Non-sensitizer for skin. Non-permeator by skin. Non-hazardous in case of ingestion. Non-hazardous in case of inhalation. Non-irritant for lungs. Non-sensitizer for lungs. Non-corrosive to the eyes. Noncorrosive for lungs ([redacted]

MODULE 2 OVERALL SUMMARIES

2.4 Nonclinical Overview

2.4.5 Integrated Overview and Conclusions

Permethrin is widely used as an insecticide, acaricide, and insect repellent. It belongs to the family of synthetic chemicals called pyrethroids and functions as a neurotoxin, affecting neuron membranes by prolonging sodium channel activation. Permethrin is a first-line treatment for scabies and is effective against crab lice. Permethrin is on the WHO's List of Essential Medicines, the most important medications needed in a basic health system.

Permethrin is extensively and rapidly metabolized following absorption. The two major pathways for metabolism are hydrolysis, essentially splitting the molecule in two, or oxidation at a number of carbon atoms throughout the molecule. Both of these metabolic processes result in a metabolite that is more water soluble than the parent compound and thus more likely to be excreted in the urine. The metabolism is an important detoxification pathway for permethrin, because it is the parent chemical that exerts its neurotoxic effects. Permethrin, following absorption, is distributed throughout the body but appears to concentrate most predominantly in the fat. This observation might explain its high concentrations in brain and nervous tissue. DEET in combination with permethrin might actually facilitate the dermal absorption of permethrin. Greater permeability of the immature BBB to *cis*- and *trans*-permethrin may contribute to the increased susceptibility of pre-weanling rodents to the insecticides. The maximal plasma and tissue concentrations occurred at 6 h after the administration. In pregnant rats with oral dosing, the highest concentrations were observed in fat and mammary glands. Binding of deltamethrin, *cis*-permethrin, and *trans*-permethrin to plasma proteins and lipoproteins was linear from 250 to 750 nM in the rat.

Permethrin is acutely toxic at high doses in animals and humans (LD_{50} for animals is greater than 1 g/kg); the toxicity varies with the *cis*/*trans* ratio – the *cis* isomer being more toxic than the *trans* isomer. Acute signs of toxicity to the central nervous system include incoordination, ataxia, hyperactivity, convulsions, and finally prostration, paralysis, and death. Permethrin can be an ocular irritant following direct application to the eye. It can also be a skin irritant and sensitizer after dermal exposure at high concentrations. There is little evidence that short-term (up to 13 weeks), repeated exposures are highly toxic to mammals; the NOEL in feeding studies of rats ranged from 20 to 1,500 mg/kg of diet in 3- and 6-month studies. Rats and mice have survived exposures as high as 10.000 mg/kg (in feed) for 2-26 weeks, although clinical signs of toxicity were clearly evident. NOELs in dogs ranged from 5 mg/kg/day to 250 mg/kg/day. In most studies, no effects were observed in haematological or serum chemistry values, even at exposure levels that produced clinical signs of toxicity. However, at near lethal doses in rats, increases in serum aspartate aminotransaminase, alanine aminotransaminase, and lactic dehydrogenase enzymes were reported that suggest some liver toxicity. The primary organ showing morphological changes is the liver. In most studies in rodents, livers were enlarged

(absolute and relative to body weight) but only at clearly toxic doses, and they returned close to normal after exposure ceased. Microscopically, hepatocellular swelling occurred, which has been attributed to increased microsomal activity resulting in a proliferation of endoplasmic reticulum. Permethrin causes significant changes in the urine metabolites indicating kidney and liver toxicity. No morphological changes in the liver of dogs were observed at exposure levels of up to 2.000 mg/kg/day, PO for 3 months, although a slight increase in liver weight was observed at doses above 50 mg/kg. No significant toxic effects were seen in rabbits administered permethrin for 10 or 28 days, respectively.

Data on the reproductive and developmental toxicity of orally administered permethrin suggest that there are few toxic effects and these tend to occur at high doses. NOAELs from the mouse and rabbit studies (400 mg/kg/day and 600 mg/kg/day, respectively) were much higher than those from the rat studies. In the three-generation studies, a small increase in buphthalmos and persistent papillary membrane was observed in weanling rats following continuous exposure to permethrin at 1.000 and 2.500 ppm (50 and 125 mg/kg per day); the NOAEL was 500 ppm (25 mg/kg/day). Studies conducted to determine the potential of permethrin to produce gene mutations in microbial and mammalian systems were all negative. Of the two *in vivo* studies conducted in the micronucleus assay, one was negative and the other was considered inadequate. Three *in vitro* studies in which clastogenicity of permethrin was investigated provided evidence of potential clastogenicity of permethrin. Small, statistically significant elevations in sister chomatid exchanges, micronuclei, and chromosomal aberrations in human lymphocyte cultures were reported. Other genotoxicity tests of permethrin (dominant lethal test and tests for DNA damage in microbial and mammalian cells) were negative. Permethrin has been tested in seven chronic carcinogenicity studies, three studies in rats and four in mice. The three rat studies were negative for carcinogenicity; however, the studies were not conducted at doses high enough to adequately assess the oncogenic potential of permethrin. Two of the four mouse studies showed evidence of carcinogenicity. Lung adenomas and carcinomas separately showed statistically significant dose-related trends.

Excipients of the formulation are approved and established agents in widespread use in the pharmaceutical manufacturing industry.

In conclusion, the pharmacodynamic data and the safety profile of permethrin obtained in animals are in line with observations made during the last thirty years of its application in human therapy, and continue to support its human application.

MODULE 2 OVERALL SUMMARIES

2.4 Nonclinical Overview

2.4.6 Literature References

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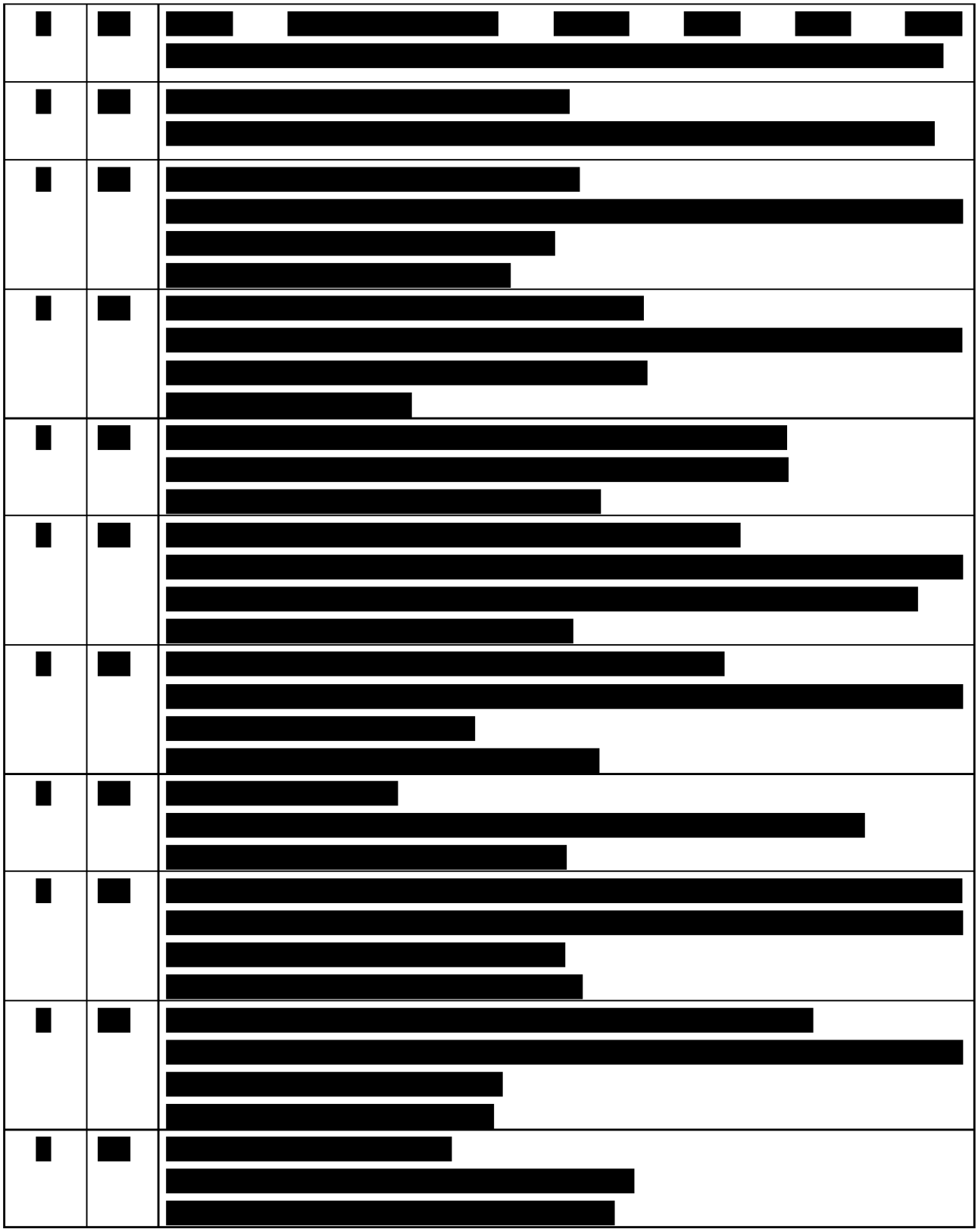

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