European Food Safety Authority

The EFSA Journal (2009) 921, 1-34

SCIENTIFIC OPINION

Potential developmental neurotoxicity of deltamethrin [1]

Scientific Opinion of the Panel on Plant Protection Products and their Residues (PPR)

(Question No EFSA-Q-2008-373)

Adopted on 9 December 2008

PANEL MEMBERS

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SUMMARY

Following a request from the Commission, the Panel on Plant Protection Products and their Residues (PPR) was asked to deliver a scientific opinion on the possible developmental neurotoxicity of deltamethrin. The potential developmental neurotoxicity of deltamethrin was addressed by the notifier in guideline-compliant reproductive and developmental toxicity studies in different animal species including a multi-generation study in rats and a separate developmental neurotoxicity (DNT) study in the rat, in accordance with the new OECD guideline TG426. The data available from the open literature include experimental studies carried out in rodents involving exposure during prenatal life and/or during neonatal life. These studies had several limitations and did not provide any clear evidence for a developmental neurotoxic effect of deltamethrin. The PPR Panel concluded that deltamethrin has been adequately tested for developmental neurotoxicity and that the available data do not indicate that deltamethrin is a developmental neurotoxic agent. The existing health-based guidance values for deltamethrin are based on neurological signs, as the most relevant critical effects observed in adult animals from different species. The lowest NOAEL of 1 mg/kg bw/day, used for risk assessment, was obtained in the 90-day and 1-year dog studies for which a 100-fold default safety factor (SF) was considered appropriate. No developmental neurotoxicity was observed at the highest dose tested of about 7 mg/kg bw/day in the DNT study complying with the new OECD guideline. This study provides a state-of-the-science evaluation of

[1] For citation purposes: Scientific Opinion of the Panel on Panel on Plant Protection Products and their Residues (PPR) on a request from the European Commission on potential developmental neurotoxicity of deltamethrin. *The EFSA Journal* (2009) 921, 1-34

developmental neurotoxicity. The results of this study provide a margin of safety of more than 600. The PPR Panel concludes that the existing health-based guidance values provide adequate protection against any potential developmental neurotoxicity of deltamethrin that would, anyhow, occur only at doses causing severe systemic toxicity.

Key words: deltamethrin, developmental neurotoxicity, risk assessment, pyrethroids, guidance values

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

Deltamethrin is an insecticide included in Annex I to Directive 91/414/EEC. In the review report for this active substance it was concluded that confirmatory data to further address concerns related to its potential developmental neurotoxicity should be generated, when internationally agreed testing protocols become available. New data have now been generated by the notifier and recently evaluated by the Rapporteur Member State (Sweden) for this substance.

In their evaluation, Sweden highlighted that the new developmental neurotoxicity study in the rat did not clarify the toxicological relevance of effects reported in the open literature and concerns remain about the study design used (i.e. the actual content of deltamethrin in the dam's milk is not known, the mouse was not tested as requested by Sweden in the Addendum to the DAR of June 2002).

The Rapporteur Member State, Sweden concluded that developmental neurotoxicity of deltamethrin could not be excluded. It was therefore proposed that an additional assessment factor of 3 should be applied, which would reduce the guidance values previously established, as follows: ADI from 0.01 to 0.003 mg/kg bw/day; ARfD from 0.01 to 0.003 mg/kg bw; AOEL from 0.0075 to 0.0025 mg/kg bw/day. These guidance values were based on neurotoxicity observed in adult dogs in 90-day and 1-year studies.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The PPR Panel of EFSA was asked for an opinion on the potential developmental neurotoxicity of deltamethrin.

The following questions were identified:

Question 1: Based on the available data, both in the published scientific literature and in the toxicological dossier submitted for inclusion in Annex I, does the PPR Panel consider that deltamethrin shows developmental neurotoxic effects?

Question 2: Has deltamethrin been assessed adequately for developmental neurotoxicity and if not what further information would be of value in this assessment?

Question 3: Do the existing health-based guidance values provide adequate protection against any potential developmental neurotoxicity of deltamethrin and if not what values would be necessary to provide such protection?

ACKNOWLEDGEMENTS

The European Food Safety Authority wishes to thank the members of the Working Group for the preparation of this opinion: Ms. Claudia Bolognesi, Mr. Alan Boobis (chair), Mr. David Coggon, Mr. Otto Meyer, Mr. Angelo Moretto, Ms. Maria Tasheva, Ms. Christiane Vleminckx and the EFSA staff members István Sebestyén, Hans Steinkellner and Judith Ricketts.

ASSESSMENT

1. Introduction

Deltamethrin is a synthetic type II pyrethroid used as a broad spectrum insecticide which acts both on direct contact and following ingestion.

1.1. Absorption, distribution, metabolism, excretion

The compound is rapidly absorbed and excreted after oral administration in rats: 19-47% in urine and 32-55% in faeces within 24 hours after dosing. Deltamethrin is rapidly and extensively metabolized in rats. The main routes of metabolism include cleavage of the ester bond and oxidation at position 4 of the phenoxy ring of the alcohol moiety. The acid and alcohol moieties are further transformed to conjugated metabolites. Unchanged deltamethrin is the major compound in faeces. Different compounds are found in urine. The radiocarbon from orally or intraperitoneally administered (^{14}CN)-deltamethrin is excreted more slowly than the radiocarbon from ^{14}Cv-deltamethrin (dibromovinyl position) or from (^{14}C-alpha)deltamethrin (benzylic position), and the concentrations of residues in tissues are higher, especially in the skin and stomach, in the latter case due to temporary retention of thiocyanate formed from released cyanide. The amounts of radiolabel retained in tissues and carcass 7 days after dosing are generally low, representing only 0.59–1.9% of the total dose administered. Fat contains the highest concentration of residues.

Deltamethrin absorption and excretion were also studied in lactating cows: deltamethrin appeared to be poorly absorbed from the gastrointestinal tract, as the concentrations in blood were low and little radiolabel was recovered in urine. The absorbed compound was extensively metabolized and excreted, with little accumulation in tissues. Some 36–43% of the total administered radiolabel was eliminated in faeces within 24 hours: most (78–82%) of the radiolabel in faeces was associated with unchanged deltamethrin. Only 4–6% of the administered dose was eliminated in urine and 0.42–1.6% was secreted in the milk. Deltamethrin was the major residue in milk.

1.2. Acute toxicity

Results from acute toxicity studies, reported in the DAR, show that the vehicle has considerable influence on the LD_{50}. Aqueous suspensions are significantly less toxic than solutions in oils, probably due to poor absorption from the former vehicle.

LD_{50} for Sprague Dawley rats given deltamethrin dissolved in corn oil were 95 and 87 mg/kg bw for males and females respectively. Clinical signs included gait alterations, repetitive convulsions, vocalization and salivation.

Sprague Dawley rats given deltamethrin by gavage in PEG 200 (LD_{50} of 67 and 86 mg/kg bw for males and females respectively) or sesame oil (LD_{50}s128 and 139 mg/kg bw for males and females respectively) showed motor incoordination, convulsions, changes in respiration, and hypomotility shortly afterwards.

When deltamethrin dissolved in peanut oil was administered to Sherman rats, LD_{50} values were 52, 31, and 50 mg/kg bw in males, females and weanling females respectively. Moderate to severe salivation and convulsions were seen at doses from 10 mg/kg bw in males, and mild

salivation was seen at doses from 10 mg/kg bw in females and from 15 mg/kg bw in weanling females.

Deltamethrin administered in aqueous suspension in 1% w/v methylcellulose was not acutely toxic to rats, since all animals at 5000 mg/kg bw survived. There were no changes in body weight, and no visible toxic effects were found either during the study or post mortem.

1.3. Short-term toxicity studies

Short term oral toxicity studies have been carried out in mice, rats and dogs. Treatment-related clinical signs (salivation, tremors, hypersensitivity, impaired locomotor activity, and reduced bodyweight and bodyweight gain and food consumption) occurred at low dose levels. The severity of the toxic effects was dependent on the vehicle used. More severe effects were observed when deltamethrin was administered by gavage as a solution in polyethylene glycol. Mortality was observed at 3000 ppm in rats (equal to 425 and 444 mg/kg bw/day in males and females respectively) and mice (equal to 603 and 739 mg/kg bw/day in males and females respectively) in 13-week dietary studies. The rat appeared to be the most sensitive species as evidenced by the severity of neurological signs at the highest doses. No treatment-related gross or microscopic changes were detected in the nervous system. The lowest NOAEL for short-term exposure (12-13 weeks) was 1 mg/kg bw/day (deltamethrin in PEG 200) and was similar in rats and dogs (Fabreguettes, 1991; Ryle et al., 1991a and b; Hunter, 1977; Chesterman, 1977, all cited in the DAR).

A sub-chronic toxicity study has been carried out in dogs. Beagle dogs (4 animals/sex/group) were treated orally for 1 year at doses of 1, 10 and 50 mg/kg bw/day of deltamethrin administered in gelatine capsules. Clinical signs such as unsteadiness of gait, splayed limbs/digits, tremor and chewing/scratching of the extremities were observed at 10 and 50 mg/kg bw/day. No treatment-related interference in reflexes or gross or microscopic changes were observed. The NOAEL was 1 mg/kg bw/day, based on altered behaviour and liquid faeces observed at 10 mg/kg bw/day (Ryle et al., 1993 cited in the DAR).

1.4. Long-term toxicity studies

Long-term effects of deltamethrin have been assessed in a 2-year and a 97-week study in mice and in two 2-year studies in rats**.**

A 2-year dietary study was carried out in Charles-River CD mice where no signs of overt toxicity were observed. Thus, the NOAEL was the highest doses tested (12 and 15 mg/kg bw/day in males and females respectively, Goldenthal, 1980a cited in the DAR).

A further 97-week dietary study carried out in the same strain of mice showed emaciation, dyspnoea and skin ulceration in both sexes at the highest doses. The NOAEL for male mice was 16 mg/kg bw/day based on skin lesions observed in males at 315 mg/kg bw/day. The NOAEL for females was 189 mg/kg bw/day based on clinical signs, emaciation and dyspnoea (Richard, 1995 cited in the DAR).

A first 2-year dietary study carried out in Charles-River CD rats at concentrations of 2, 20 and 50 ppm deltamethrin (in corn oil, equal to 0.1, 0.8 and 2.1 mg/kg bw/day in males and 0.1, 1.1 and 2.8 mg/kg bw/day in females) did not show any significant changes in behaviour and appearance. A significant decrease in body weight gain was observed at the highest dose in both sexes, and an increase in the mean weights of the uterus, adrenals, thyroid and pituitary in females and of testes in males was observed. At the interim kill at 18 months, an increased incidence and/or relative severity of axonal degeneration was observed in the sciatic, tibial and

plantar nerves of a small number of animals receiving 20 and 50 ppm. However, at the end of the study the incidence was comparable for both control and experimental groups (Goldenthal, 1980b cited in the DAR).

A further 2-year dietary study in rats, using higher concentrations (25, 125, 500, and 800 ppm equal to 1, 5, 22, 36 and 2, 7, 30, 47 mg/kg bw/day in males and females respectively) of deltamethrin, showed neurological signs characterized by uncoordinated movements at the highest dose in both sexes and also in males at 500 ppm. Reduced body weight and food consumption and changes in haematological parameters were also observed. Histopathologically, there was no evidence of any treatment-related neurological lesions after 52 weeks or at termination. The NOAEL in this study was 1 and 30 mg/kg bw/day in males and females respectively (Ryle, 1995 cited in the DAR).

After long-term exposure of rats and mice to deltamethrin, no carcinogenic effects were reported.

2. Re-evaluation of deltamethrin developmental neurotoxicity

2.1. Studies provided by the notifier

2.1.1. Acute neurotoxicity studies

An acute neurotoxicity study with COBS Wistar rats was performed in which the neurotoxicological signs were evaluated using a non-standard test, the “tilting plane”, without following any standard guideline. Deltamethrin (purity not stated) in corn oil was administered by gavage to groups of five male and five female Wistar rats at a dose of 0 or 25 mg/kg bw/day on 2 consecutive days. Two treated males died after the second treatment. The surviving animals were subjected to a tilting plane test every second day from day 4 to day 16 of the study. The tilting plane test entailed placing the animals on a clean dry steel platform which was tilted at a constant rate (7.5 degrees/second). The angle of inclination at which the animals begin to slide down the slope was measured and the mean of 4 trials was calculated. No neurological effect was found on the slip angle (Davies et al., 1983 cited in the DAR).

A subsequent acute neurotoxicity study was carried out in Sprague-Dawley rats. Deltamethrin (purity, 99.2%) in corn oil was administered once by gavage to groups of 12 male and 12 female rats at doses of 0, 5, 15, and 50 mg/kg bw, and the animals were tested in a "functional observation battery" before treatment and 3 h, 7 days, and 14 days later. The rats were killed on day 15. All rats were examined for viability twice a day and for clinical signs once a day. The battery of tests consisted of observations (posture, convulsions, tremors, biting, eyelid closure, and faecal consistency) in the home cage; observation of ease of handling, lachrymation, chromodacryorrhoea, piloerection, eyelid closure, red or rusty deposits, eye prominence, salivation, fur appearance, respiratory rate and character, colour of mucous membranes, eyes, and skin, and muscular tone; observation of mobility, rearing, convulsions, tremors, grooming, bizarre or stereotypical behaviour, time to first step, gait, arousal, urination, defecation, gait score, and backing in the open field; sensory observations (approach, startle, touch, tail pinch, hind limb, eye blink and pupillary responses, olfactory orientation, forelimb extension, and air righting reflex); neuromuscular observations (hind limb extensor strength, hind limb foot splay, grip-strength of forelimb and hind limb, rotarod performance) and physiological observations (catalepsy, body temperature, body weight). In addition, locomotor activity was assessed in all rats before treatment and 3 h, 7 days and 14 days after treatment. All surviving rats were killed,

and five rats from each sex per group were then perfused *in situ* for neuropathological examination of selected central and peripheral nervous system tissues.

One male and one female at 50 mg/kg bw died on the day of administration. All six of the functional domains i.e. sensorimotor, autonomic, neuromuscular, physiological, activity, and excitability, were affected by deltamethrin at 50 mg/kg bw. The effects peaked 3 hours after treatment and were transient. A statistically significant reduction in mean body weight gain was observed in males at 50 mg/kg bw. No treatment-related effects were apparent on brain weight or brain dimensions. One male animal receiving deltamethrin at a dose of 50 mg/kg bw had digestion chambers (vacuolated structures containing remnants of axonal and myelin material as a sign of ongoing demyelination) in the sciatic nerve (with axonal degeneration) and tibial nerve. One female animal receiving deltamethrin at a dose of 50 mg/kg bw had digestion chambers in the sciatic and peroneal nerves. The effects noted were recorded as minimal to mild and were in the range of values observed in historical controls. Therefore, they were considered spontaneous and unrelated to the administration of deltamethrin. One male and one female receiving 15 mg/kg bw showed potentially treatment-related signs when tested on day 0, including slight salivation in the male and slightly soiled fur in the female during handling and slightly impaired mobility of the male during open field observations. No neuropathological evaluation was performed on samples from nervous tissues from the intermediate and low dose groups. The NOAEL for neurotoxicity was 5 mg/kg bw, based on effects in the functional observation battery and on locomotor activity at 15 mg/kg bw (Nemec, 1998a cited in the DAR).

2.1.2. Short-term dietary neurotoxicity study

Deltamethrin (purity 99.2%) was administered in the diet to rats of the Crl:CD (SD) BR strain (10 animals/sex/group) at concentrations of 0, 50, 200 and 800 ppm (equal to 0, 4, 14 and 54 mg/kg bw/day for males and 0, 4, 16 and 58 mg/kg bw/day for females) for a period of 13 weeks. Experimental parameters recorded for all animals included viability, clinical signs, body weight and food consumption. The animals were subjected to the same functional observational battery of tests as described above and to observations of locomotor activity before treatment and during weeks 4, 8 and 13 after treatment. Brain weight (excluding olfactory bulbs) and brain dimensions were recorded for each animal. A neuropathological evaluation was performed on five animals/sex in the control and 800 ppm (54 mg/kg bw/day) groups.

Treatment-related deaths occurred in animals receiving deltamethrin at a concentration of 800 ppm (three male and two female rats died). Treatment-related clinical signs were limited to animals at this dose, the most prevalent being gait alterations (rocking, lurching or swaying, walking with the hindlimbs splayed, walking on tiptoes and/or writhing), hypersensitivity to noise and impaired righting reflex. Clinical signs that occurred less frequently included piloerection, convulsions, "popcorn seizures", altered posture (flattened with limbs extended), and tan staining of the fur. The functional observational battery revealed changes in animals at 800 ppm, often at all three times (weeks 3, 7, and 12), which included piloerection and slightly soiled fur in the home cage; impaired mobility and gait, and bizarre or stereotypic behaviour (rocking side-to-side) in the open field; altered air-righting reflex; altered hind limb extensor strength; and reduced forelimb and hindlimb strength. No treatment-related effect was seen on locomotor activity. Hypersensitivity to noise and gait alterations were also observed in males and females at 200 ppm. The mean body weights of males and females at 800 ppm were decreased during weeks 1–13, primarily due to low mean body-weight gains or mean body-weight losses during the first 3 weeks of the study. The food consumption of animals at this dose was also reduced throughout the study. No treatment-related changes in brain weight or

dimensions were observed. Microscopic examination of perfused tissues (including sciatic, sural, tibial and peroneal nerves) from animals at 800 ppm revealed no treatment-related neuropathological lesions. The NOAEL for systemic toxicity and neurotoxicity was 50 ppm, equal to 4 mg/kg bw/day, based on clinical signs noted in animals at 200 ppm, equal to 14 mg/kg bw/day (Nemec, 1998b cited in the DAR).

2.1.3. Reproductive toxicity

Deltamethrin did not affect reproduction. No treatment related embryotoxic or teratogenic effects were observed in mice, rats and rabbits.

2.1.3.1. Two-generation reproductive toxicity study

Deltamethrin (purity 99.7%) was administered in the diet to groups of 30 Charles River Crl:CD BR VAF/Plus rats of each sex at concentrations of 0, 5, 20, 80 and 320 ppm. The F_0 rats were exposed for 12 weeks before the 3-week mating period and then throughout gestation and lactation; they were killed on day 21 after birth of the F_1 generation. Whenever possible, at least one pup of each sex was selected on day 21 after birth for continuation in the study. These animals were treated the same as the F_0 rats and killed after production of the F_2 litters. Clinical signs, body weights, food consumption, mortality, mating and fertility, rearing capacity, natural delivery, litter observations and observations at necropsy were recorded. Necropsy also included brain examination for hydrocephaly.

The death of one F_0 female rat at 320 ppm was attributed to treatment, as the animal showed gastric erosions similar to those found in a dose range-finding study. Significant numbers of animals in this group showed clinical signs attributable to deltamethrin. In particular ataxia and hyperactivity, vocalization, and excessive salivation occurred in the females during lactation. Body weight gain, terminal body weight and food consumption of animals at this dose were reduced, whereas the organ:brain weight ratios were unaffected. Neither the mating performance nor the fertility of the F_0 generation was affected, but the pups of F_0 parents at 320 ppm weighed less at birth and significantly less ($p \leq 0.01$) on days 4, 7, 14 and 21 *post partum*; the mortality rate among these pups was significantly increased ($p \leq 0.01$) on days 8 and 14 *post partum*, and the lactation index was consequently reduced. In the F_1 generation of rats at 320 ppm, deaths of 17 males ($p \leq 0.01$) and 19 females ($p \leq 0.01$) were attributable to deltamethrin. Most of the deaths occurred within 8 days of weaning. Before death, many of these animals showed ataxia, impaired righting reflex, vocalization and excess salivation. Many of the remaining surviving animals at this dose showed similar clinical signs. One of the male rats that died had gastric erosions. No clinical signs occurred in significant numbers of rats in the other dose groups. Body weight, body weight gain and food consumption were significantly reduced ($p \leq 0.05$ to $p \leq 0.01$) in rats at the highest dose throughout the period before mating and, in female rats, during gestation and lactation. Neither the mating performance nor the fertility of F_1 rats was affected, but the pups of F_1 rats at 320 ppm weighed significantly less than controls ($p \leq 0.05$ to $p \leq 0.01$) on days 7, 14 and 21 *post partum*. No other biologically important differences were found between the groups in terms of pup viability, sex ratio, or clinical or autopsy observations. The NOAEL for parental toxicity was 80 ppm, equal to 4.2 mg/kg bw/day based on clinical signs (ataxia, hypersensitivity) in females during gestation and lactation and reduced body-weight gain and feed consumption with increased mortality rates at 320 ppm, equal to 18 mg/kg bw/day. The NOAEL for offspring toxicity was also 80 ppm, equal to 11 mg/kg bw/day, based on reduced body weight, clinical signs (ataxia, impaired righting reflex, hyperactivity, splayed limbs), reduced viability and increased mortality rates

before and after weaning up to 18 days. The NOAEL for reproductive toxicity was 320 ppm, equal to 18 mg/kg bw/day, the highest dose tested (Hoberman, 1992 cited in the DAR).

2.1.3.2. Developmental toxicity studies

Developmental effects of deltamethrin have been assessed in one mouse study, two rat and two rabbit studies.

Deltamethrin (purity not stated) dissolved in corn oil was administered by gavage to groups of 30 inseminated CD-1 mice at a daily dose of 0, 3, 6, or 12 mg/kg bw on days 7–16 of gestation. The mice were killed on day 18 of gestation. A dose-related reduction in maternal weight gain during gestation was observed, animals at the high dose gaining 42% of the weight of controls. The mortality rate was not affected by dose, but convulsions were observed in dams at the two higher doses. Treatment did not affect the number of implantation sites, foetal weights, or the number of sternal and caudal ossification centres. A significant ($p < 0.01$), dose-related increase in the occurrence of supernumerary ribs was observed, from 13% in the controls to 23, 47, and 28% at the three increasing doses. No other treatment-related skeletal or visceral anomalies were observed. The NOAEL for maternal toxicity was 3 mg/kg bw/day based on reduced body weight gain and convulsions at 6 mg/kg bw/day, but no NOAEL for developmental toxicity could be determined due to the increased incidence of supernumerary ribs in mice in all the treated groups (Kavlock et al., 1979a cited in the DAR).

Deltamethrin (purity not stated) was dissolved in corn oil and administered by gavage to groups of 30 inseminated Sprague-Dawley rats at daily doses of 0, 1.2, 2.5, and 5.0 mg/kg bw on days 7–20 of gestation. The rats were killed on day 21 of gestation. The body-weight gain of animals at the highest dose was reduced to 80% of that of controls. No effects were observed on the number of implantation sites, foetal mortality, foetal weight gain or the number of sternal and caudal ossification centres. The NOAEL for maternal toxicity was 2.5 mg/kg bw/day based on reduced body weight gain and mild salivation at 5.0 mg/kg bw/day. In the absence of malformations and developmental variations in the foetuses at the highest dose tested, the NOAEL for neonatal toxicity was 5.0 mg/kg bw/day (Kavlock et al., 1979b cited in the DAR).

Deltamethrin (purity 99.2%) dissolved in corn oil was administered by gavage to groups of 25 gravid Charles River Crl:CD VAF/Plus rats on days 6–15 of gestation at initial doses of 0, 1, 3.3, and 11 mg/kg bw/day. Owing to excessive toxicity, a group receiving 7 mg/kg bw/day was added and subsequently, because of unacceptable concentration analyses, additional groups given 0, 1, and 3.3 mg/kg bw/day were added. The foetuses were removed from all surviving females on day 20 of gestation and were examined. The mortality and moribundity rates of dams at 7 and 11 mg/kg bw/day were significantly elevated. No maternal effects were observed in the groups given 1 and 3.3 mg/kg bw/day. No reductions were found at any dose in the numbers of pregnant females, viable foetuses per pregnancy or total implants per pregnancy. Foetal body weights were not affected by treatment and the male:female ratio was unchanged. In comparisons with the original control group, the incidence of soft tissue or skeletal malformations was not increased in rats at 1 or 11 mg/kg bw/day, but abnormalities were found in those dosed at 3.3 mg/kg bw/day. These consisted mainly of folded retina, vertebral malformations and fused sternebrae. Similar malformations were not observed in the additional group at 3.3 mg/kg bw/day, although they did occur in the additional control group. Hence, these observations were considered unrelated to treatment. The NOAEL for maternal toxicity was 3.3 mg/kg bw/day based on clinical signs (moribundity, convulsions, increased salivation, hypersensitivity, staining), reduced body weight gain (throughout treatment at 7 and 11 mg/kg bw/day and during gestation at 11 mg/kg bw/day) and deaths. In the absence of malformations

and developmental variations in foetuses at the highest dose tested, the NOAEL for developmental toxicity was 11 mg/kg bw/day (Schardein, 1990 cited in the DAR).

A developmental toxicity study was carried out in New Zealand White (NZW) rabbits with deltamethrin (purity 99.4%) suspended in Tween 80 and diluted in 0.5% carboxymethylcellulose at dose levels of 0, 10, 25 or 100 mg/kg bw on days 7–19 of gestation. One animal died at 100 mg/kg bw, which was considered to be compound-related. The NOAEL for maternal toxicity was 25 mg/kg bw/day based on the death of one female. The NOAEL for developmental toxicity was also 25 mg/kg bw/day, based on retardation of ossification at 100 mg/kg bw/day. No evidence of teratogenic effects was found. The low toxicity of the compound in this study could be related to the choice of the vehicle for administration: a suspension in carboxymethylcellulose instead of a solution in corn oil. This study was not considered acceptable for risk assessment because it was questionable whether adequate intake had occurred (Schardein, 1990 cited in the DAR).

A new developmental toxicity study in rabbits was submitted in 2001. Female rabbits of the KBL New Zealand White strain (24 animals/dose level) were treated by gavage with deltamethrin (purity 99.1%) dissolved in corn oil at dose levels of 0 (control), 3, 10 or 32 mg/kg bw/day from day 6 to day 28 *post-coitum*, inclusive. The control animals received the vehicle (corn oil) only. On day 29 *post-coitum*, the does were killed, the gravid uterus was weighed, and a macroscopic post-mortem examination performed. The foetuses were removed by caesarean delivery. All the foetuses were weighed and subjected to an external examination. No deaths, abortions or clinical signs that were considered to be related to the toxicity of the test substance were observed in any group. Decreased food consumption (not statistically significant, 22% decrease) and lower body weight gain (not statistically significant, 68% decrease) were noted for females in the highest dose group when compared to the control group. No treatment-related effects were observed on the number of corpora lutea, the number of implantation sites, malformations or alterations in foetuses. The NOAEL for maternal toxicity was 10 mg/kg bw/day based on decreased food consumption and body weight gain in animals from the highest (32 mg/kg bw/day) dose group. The NOAEL for developmental toxicity was 32 mg/kg bw/day, the highest dose tested (Richard, 2001 cited in the Addendum to the DAR).

2.1.3.3 Developmental neurotoxicity study

A developmental neurotoxicity (DNT) study in accordance with the new OECD guideline TG 426 (OECD, 2007) was carried out in Wistar rats, in response to the requirement set in the inclusion directive for deltamethrin (as for other pyrethroids). Deltamethrin technical grade was administered to 30 mated female rats via the diet at nominal concentrations of 0, 20, 80 and 200 ppm corresponding to mean daily intakes of 0, 1.64, 6.78 and 16.1 mg deltamethrin/kg bw/day, from gestation day (GD) 6 through to lactation day (LD) 21. A functional observational battery (FOB) was performed on all dams on GDs 13 and 20 and on LDs 11 and 21. Offspring (minimum 10 pups/sex/dose) were evaluated using the following observations and measurements: detailed clinical observations and a functional observational battery, preputial separation or vaginal patency, body weight, automated measures of activity (figure-eight maze), auditory startle habituation, learning and memory (passive avoidance after weaning and a water maze task beginning on PND 60±2 days) and an ophthalmic examination. Neural tissues were collected from 10 animals/sex/dietary level on PND21 and at study termination (approximately 75 days of age) for microscopic examination and morphometry.

A pilot study was conducted to verify deltamethrin exposure of the offspring during lactation (PND10 to 16) and to determine how well Wistar rats would tolerate exposure to a dietary concentration of 250 ppm from GD6 through day 16 of lactation. In this study, a dietary concentration of 250 ppm in mated female Wistar rats caused an increased incidence of pup loss (incidence not specified) including cannibalization by the dam during the first week of lactation indicating toxicity to either the dam or offspring. Deltamethrin was determined in brain tissue from the first three male and three female offspring in each litter (one/sex/litter at each age) at PND10, 14 and 16. Mean levels of deltamethrin were 34.7 ± 7.7, 37.2 ±7.5 and 32.1 ± 5.0 ppb, respectively. These results demonstrate that the potential target organ of the offspring was exposed to deltamethrin during lactation.

In dams treated at 20 and 80 ppm, there were no treatment-related findings during gestation or lactation. Maternal effects (reduced body weight (6-7%) and bodyweight gain (17%), and reduced food consumption) were noted at 200 ppm (16.1 mg/kg bw/day). Reproductive parameters were not affected by the test substance at any dietary level. Effects in the offspring (reduced pre-weaning body weight (males: 10%, females: 9%), reduced bodyweight gain (males: 15%; females: 18%), increased incidence of vocalizations with handling in males only on PND4 and a statistically significant delay in balanopreputial separation (45.1 days vs. 43.5 days for controls) were noted at 200 ppm (16.1 mg/kg bw/day). The toxicological significance of the isolated finding of vocalization on handling of males on PND4 is not clear. The maternal NOAEL was 80 ppm (equal to 6.78 mg/kg bw/day) based on reduced bodyweight gain (>10%) in dams at 200 ppm (equal to 16.1 mg/kg bw/day). The NOAEL for the offspring was 80 ppm based on reduced bodyweight gain (>10%) and delayed balanopreputial separation at 200 ppm (Gilmore et al., 2006 cited in the New Annex II).

2.2. Experimental studies from the open literature

2.2.1 Developmental neurotoxicity studies in mice

Table 1 summarizes results from developmental neurotoxicity studies in mice.

Deltamethrin (purity not specified) was administered orally by a PVC tube at concentrations of 0.71 and 1.2 mg/kg bw/day for 7 days to ten day old NMRI mice of both sexes. The substance was dissolved in a mixture of egg lecithin and peanut oil (1:10, w:w) and was thereafter sonicated together with water to yield a 20% (w:w) fat emulsion. The mice were killed by decapitation 24 h after the last administration and crude synaptosomal fractions (P2) were prepared from the cerebral cortex and hippocampus. The muscarinic receptor density was determined by measuring the amount of tritium-labelled quinuclidinyl benzilate ([^{3}H]QNB) specifically bound in the P2 fraction using atropine to measure the non-specific binding (11-18 analyses/group using P2 fractions pooled from two animals were used in this measurement). The proportions of muscarinic high and low affinity binding sites were assessed using [^{3}H]QNB/carbachol (8-9 analyses/group using P2 fractions pooled from two animals were used in this measurement). Measurements of the high-affinity nicotinic binding sites were performed using tritium-labelled nicotine (6-15 analyses/group using P2 fractions pooled from two or four animals for cortex and hippocampus, respectively). Data were presented for male and female mice combined and not separately by sex.

Mice receiving the higher dose of deltamethrin (1.2 mg/kg bw) developed choreoathetosis (involuntary movements) 1 hour after administration. The signs persisted for about 5 h on the first day, 3 h on the second and disappeared on the fourth day. At this dose, a decrease (7%) in the density of muscarinic receptors in the hippocampus and an increase (10%) in the density of

nicotinic receptors in the cerebral cortex were observed. The lower dose (0.71 mg/kg bw/day) did not cause any clinical signs but increased the muscarinic receptor density (8%), high affinity sites increasing by 45% relative to the controls, and in the nicotinic receptor density (21%) in the cortex (Eriksson and Nordberg, 1990).

Deltamethrin (purity not specified) was administered orally via a PVC tube at a dose of 0.7 mg/kg bw/day for 7 days to 10-day-old NMRI mice. The substance was dissolved in a mixture of egg lecithin and peanut oil (1:10, w:w) and was thereafter sonicated together with water to yield a 20% (w:w) fat emulsion. Each treatment group consisted of 12 mice from three different litters. Spontaneous behaviour was tested in male mice at the age of 17 days (only locomotion was measured) and 4 months (when locomotion, rearing and total activity were measured) (12 male animals/group). Motor activity was measured once, over a period of 60 minutes, divided into 3 x 20 minutes epochs in an automated device consisting of cages (40x25x15 cm) placed within two series of infrared beams forming a low level and high level grid. Locomotion counting occurred when the mouse moved horizontally through the low-level grid of infrared beams. Rearing was registered as vertical movement at a rate of four counts per second, as long as a single high-level beam was interrupted. Total activity was registered by a sensor with which the test cage was in contact. The sensor registered all types of vibration within the test cage. The mice were killed by decapitation after the test for behavioural changes at an adult age of about 4 months. Crude synaptosomal fractions (P2) were prepared from the cerebral cortex, hippocampus and striatum. Measurements of muscarinic cholinergic receptor density and of the proportions of high- and low- affinity binding sites of the muscarinic receptors were performed using the methods described for the previous paper.

No clinical signs of acute toxicity were observed in the treated mice throughout the experimental period. Body weight gains were similar for control and pyrethroid-treated animals. In the 17-day-old mice treated with deltamethrin between PND10 and PND16 there was no statistically significant change in the behavioural variable (only tested for locomotion) compared to the control group. On the other hand, in the 4-month-old mice a statistically significant increase ($p \leq 0.01$) was observed in locomotion and total activity during the last 20-minute period. A tendency towards a decrease (5%, $0.05 \leq p \leq 0.1$) in the density of muscarinic receptors in the cerebral cortex was observed in this group of mice without any change in the proportions of high- and low-affinity binding sites. In hippocampus and striatum, no effect was observed (Eriksson and Fredriksson, 1991).

A further study with the same experimental design was reported. Deltamethrin (purity 100%) was administered orally via a PVC tube at a daily dose of 0.7 mg/kg bw/day for 7 days to 10-day-old NMRI mice. One experiment was carried out using the substance dissolved in a mixture of egg lecithin and peanut oil (1:10, w:w) and then sonicated together with water to yield a 40% (w:w) fat emulsion. Two additional experiments were performed, in which the substance was administered dissolved in corn oil. Each treatment group consisted of male mice from at least three different litters. Spontaneous behaviour (mobile activity and rearing) was assessed at the age of 4 months (at least 12 animals/group). Motor activity (slow static counts, fast static counts, total static counts, slow mobile counts, fast mobile counts, total mobile counts, mobile time, slow rearing counts, fast rearing counts, total rearing counts and rearing time) was measured once over 3 successive 20 minute periods by an automated device consisting of cages (42x22x20 cm) placed within two series of infrared beams forming low level and high level grids. Approximately 1 week after the behavioural tests or 24 h after dosing at the age of 17 days, mice were killed by decapitation. Crude synaptosomal fractions (P2) were prepared from the cerebral cortex. Muscarinic receptor density was measured in the cerebral cortex (5-8 male mice/group).

No clinical signs of acute toxicity were observed in the treated mice throughout the experimental period. Body weight gains were similar for control and pyrethroid-treated animals. A statistically significant increase in rearing time, fast mobile counts, total mobile counts and in slow, fast and total rearing counts, and a statistically significant delay in habituation of slow mobile counts and mobile time were observed at the age of 4 months in male mice treated with the substance dissolved in the emulsion (egg lecithin:peanut oil 1:10 40% in water). No measurement of muscarinic receptor density was carried out in this group. A statistically significant delay in habituation of slow mobile counts, and in mobile and rearing time, without any effect on muscarinic receptor density in cerebral cortex was evident in only one of the two experiments using corn oil as a solvent. In the other experiment no behavioural alteration was detected, but a statistically significant decrease (14%) in muscarinic receptor density in the cerebral cortex was observed. In 17-day old mice treated with the substance dissolved in corn oil, a statistically significant increase (19%) in muscarinic receptor density was observed in the cerebral cortex (Muhammad and Ray, 1997 cited in the DAR and in Shafer et al., 2005).

Table 1 **Developmental neurotoxicity studies in mice**

Strain	**Treatment**	**Test**	**Clinical signs**	**Reference**
NMRI mice ten days old (8 mice per treatment)	1.2 (2.4 µmol)/kg bw/day for 7days (PND10-PND16) Vehicle: Egg lecithin: peanut oil 1:10 20% in water Orally via a PVC tube 0.71 (1.4 µmol)/kg bw/day for 7days	**Muscarinic receptor density** **Cortex**: no effect **Hippocampus**: decrease (7%) **Nicotinic receptor density** **Cortex**: increase (9 %) **Hippocampus**: no effect **Muscarinic receptor density** **Cortex:** increase (8%) High affinity sites: increase (33.5 % vs. 23.5%) Low affinity sites: decrease (66.5% vs. 76.9%) **Hippocampus**: no effect **Nicotinic receptor density** **Cortex** increase (21%)	**Choreoathetosis** 1 h after admin (5 h day 1, 3h day 2, slight signs day 3) **No clinical signs**	Eriksson and Nordberg, 1990
NMRI mice ten days old 12 mice per treatment group	0.7 (1.4 µmol)/kg bw/day for 7days PND10-PND16 Egg lecithin: peanut oil 1:10 20% in water Orally via a PVC tube	17 day-old mice Adult mice 4 month-old **Muscarinic receptor density**: Cortex: tendency to decrease (1207 vs. 1266) Hippocampus and striatum**:** no effect	**No effect** **Disruption of the habituation:** hyperactive condition	Eriksson and Fredriksson, 1991

NMRI mice ten days old (8 mice per treatment)	0.7 (1.4 µmol)/kg bw/day PND10-PND16 Vehicle: Egg lecithin: peanut oil 1:10, 40% in water Orally via a PVC tube Vehicle: Corn oil .	**Muscarinic receptor density in cortex:** 4- month old mice: Not measured. 4- month old mice: Exp 1: No effect 17-day old mice: increase (19%) 4- month old mice: Exp 2: decrease (14%)	**Activity (mobile and/or rearing):** increase **Habituation (mobile and/or rearing):** decrease **Habituation (mobile and/or rearing):** decrease **No effect** **Not determined**	Muhammad and Ray, 1997

2.2.2. Developmental neurotoxicity studies in rats

The results of developmental neurotoxicity studies in rats are summarized in Table 2.

A study was carried out in Wistar rats to investigate the long-lasting effects of prenatal exposure of dams from gestation day (GD) 6 to GD15 to a dose of a deltamethrin formulation that did not induce maternal toxicity. Nine pregnant females were treated once a day at 0.08 mg/kg in 1 ml/kg vehicle. The control group (n = 8) was similarly treated, but with the vehicle alone (1 ml/kg, 1:50 solution, formulation not revealed, w/v). Reflexes (surface righting reflex, negative geotaxis and palmar grasp) and open field behaviour (locomotion test and rearing) were evaluated in offspring at postnatal day 21 (PND1); forced swimming and open-field behaviour were examined at PND60. Noradrenaline (NA), dopamine (DA) and their metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) and serotonin (5-HT) and its metabolite 5-hydroxyindolacetic acid (5-HIAA) were measured in corpus striatum. Prenatal exposure to deltamethrin did not affect maternal weight.

An increase of rearing in males was observed at PND21. A decrease in male locomotion frequency and in immobility time in the swimming test were detected in males at PND60, but no difference was observed in male float duration. Female rats prenatally exposed to deltamethrin did not show alteration in either parameter of swimming behaviour. No acute depressive effect was observed on prenatal exposure to deltamethrin and behavioural deficits in float duration were not observed. An increase in NA, DOPAC and DOPAC/DA concentrations was observed in males without any change in DA or HVA concentrations. Changes in catecholamine and specially brain NA levels have been associated with behavioural deficits caused by acute stress exposure. The authors concluded that the behavioural changes were probably related to the increase in NA observed in this study (Lazarini et al., 2001).

A further study is available on the evaluation of long lasting effects on neurobehavioural and neurochemical parameters during adult life determined by prenatal exposure to deltamethrin at doses that were not maternally toxic. Pregnant female Wistar rats were exposed to deltamethrin formulation (Decis 2.8% emulsified concentrate (EC), 2.8% of technical grade deltamethrin (w/w, 1.0 mg/kg bw/day) by oral gavage from GD14 to GD20, the period of early neurogenesis. From PND1 to PND21 pups in both groups were examined daily using a functional test battery. Shock-motivated visual discrimination response in a Y maze was used to evaluate learning and memory performance. Six pups each of each sex were randomly selected from the control and deltamethrin-treated groups and killed by decapitation at 6 and 12 weeks of age. Brains and hippocampi were removed for determination of AChE activity in the

microsomal fraction. Muscarinic receptor density was measured in the hippocampus (6 animals/sex/group). GAP-43 (growth-associated protein) expression was evaluated in brain by an immunohistochemical method.

No significant differences were observed in the body weight of treated pups, monitored postnatally, in comparison to control pups. No gross abnormalities or developmental effects were observed. A decrease in relearning index was observed in deltamethrin-treated animals at 6 and 12 weeks of age. The relearning index, assessed by calculating the percent change in incorrect runs, decreased significantly (mean values 28 and 22.5 compared to values in controls of 38 and 33 at 6 and 12 weeks respectively). An increase in acetylcholinesterase (AChE) activity in the hippocampal region (28% and 16% above controls) and a decrease in muscarinic receptor density (48% and 39% of controls at 6 and 12 weeks, respectively) were observed. A decrease in the level of growth-associated protein (GAP-43), a neuron-specific protein present in the axonal growth cone and a marker for neuronal differentiation and synaptogenesis, evaluated as immunoreactivity in terms of % area, was observed at 6, and to a lesser extent, at 12 weeks. The authors concluded that prenatal exposure to deltamethrin causes a delay in normal development and outgrowth of neurons in exposed progeny at 6 and 12 weeks evident from neurochemical and immunohistochemical studies. The overexpression of GAP-43 in exposed animals revealed impairment in the maturity of neurons in the hippocampus region (Aziz et al., 2001).

The effects of deltamethrin on the morphology of the cerebellum were investigated in rats. Sprague Dawley rats were treated with deltamethrin 0.7 mg/kg i.p. in propylene glycol from postnatal day 9 to PND13. Four animals/group were analysed. The animals were weighed and dissected on PND12, 15, 21 and 30. The brains were carefully dissected and processed for histological analysis. About 97% of the total cell number in the cerebellum of the rat is formed after birth. During this period some of the key events in cerebellar development occur e.g., granule cell proliferation and migration, formation of the cells of the molecular layer, Purkinje cell arborisation, spine formation and synaptic formation. In the control animals the maximum cellularity of the molecular layer was observed in the PND15 pups and the external granular layer had disappeared completely in the PND21 pups. The animals exposed to deltamethrin had a lower molecular layer thickness as compared to their controls. The external granular layer was seen to be persistent and to present several mitotic figures in the PND21-treated animals. A delay in the appearance of the monolayer arrangement of Purkinje cells was observed in PND 15-treated and even, at some sites, in PND21-treated animals, suggesting a general retardation of histogenesis and morphogenesis of the cerebellum. The continued migration of granule cells in exposed animals until PND21, added further to the evidence that deltamethrin delays and/or restricts differentiation of micro-neurons in the cerebellum. In the deltamethrin-treated animals starting from PND12, major changes in the vasculature of the cerebellum were also observed. The PND15 treated animals had aggregates of blood cells in their capillaries, which in sections appeared to be clots and formed thrombi. Such clots were recorded even in PND21 and PND30 treated pups (Patro et al., 1997).

The neurotoxic effects of repeated doses of deltamethrin were investigated in young Wistar rats. Deltamethrin (Decis 2.8% in corn oil) was administered by gavage at a dose of 7 mg/kg bw/day from PND22 to PND37. A statistically significant increase in spontaneous locomotor activity (20%) and an impairment of learning performance were observed 24 hours after the last administration. An increase in the activity of monoamine oxidase (MAO) of 90% and of acetylcholinesterase of 15% was also detected in whole brain of treated rats. In addition deltamethrin induced differential effects in the levels of polyamines (putrescine, spermidine, spermine) in different brain areas. Levels of the three polyamines were significantly increased

in the hypothalamus by 46%, 44% and 49% over the controls, while in frontal cortex only spermidine and spermine levels were increased (by 79% and 100% respectively). The other brain regions, such as pons medulla and hippocampus showed a significant decrease of spermidine and spermine (Husain et al., 1994).

Table 2. **Developmental neurotoxicity studies in rats**

Strain	Treatment	Test	Clinical signs	Reference
Wistar rats 9 pregnant females Controls: 8 pregnant females	GD6-GD15 0.08 mg/kg bw/day 1 ml/kg vehicle not specified 1:50 solution	DOPAC, DOPAC/DA, NA males: increase 5HT, 5HIAA, HVA/DA: no effect	PND21: **Reflexes** (palmar graft, surface righting reflex, Negative geotaxis): **no effect** **Locomotion: no effect** **Rearing frequency** males: increase PND60: **Locomotion** males: decrease **Swimming behaviour latency time** male: decrease	Lazarini et al., 2001
Wistar rats 10 pregnant females per group	GD14-GD20 1 mg/kg bw/day Deltamethrin formulation 2.8% Oral gavage in corn oil 6 animals/sex/ group	PND42: **AChE in hippocampal region:** increase 28% **Muscarinic receptors:** decrease 48% over expression of **GAP-43** (Immunoreactivity: % area) (25.7 vs. 4.46) PND84 **AChE:** decrease 16% **Muscarinic receptors:** decrease 39% **over expression of GAP-43** (14.31 vs. 3.12)	**Shock motivated visual discrimination response (Ymaze):** decrease **Reduced relearning index (% change in incorrect runs):** 28 vs 38 **Reduced relearning index:** 22.5 vs 33	Aziz et al., 2001
Wistar rats 4 animals per group	PND9-13 0.7 mg/kg bw/day i.p. in propylene glycol	PND12, PND15, PND21, PND30	**Body weight and brain weight**: decrease **Delayed cerebellar morphogenesis of interneurons** **Vascular damage with focal degeneration**	Patro et al., 1997

Wistar rats 50 animals per group	PND22-37 7 mg/kg bw/day by oral gavage deltamethrin formulation 2.8% in corn oil	PND38 **increase of MAO** (90%) **and AChE activity** (15%) :	PND38 **Spontaneous locomotor activity:** increased by 20% **Learning performance:** reduced by 26%.	Husain et al., 1994

The age-dependent neurotoxicity of deltamethrin was investigated using immature and adult Long Evans rats (Table 3). Primiparous dams were received one week before parturition. On postnatal day 1, pups from all litters were randomized and redistributed, with each dam assigned 8 pups. Deltamethrin (purity 97%) was administered by gavage in corn oil to weanling (21 day old) and adult rats. One male and one female from each of 12 litters were treated with 0, 1, 2 or 4 mg/kg bw. Adult rats (8/dose level) received doses of 0, 2, 4 or 6 mg/kg. ASR (acoustic startle response), a measure of sensorimotor function, was selected as a behavioural test. Brain concentration of deltamethrin was measured. A dose-related decrease in ASR amplitude was observed in weanling rats (18 %, 39%, 59% for doses of 1, 2, and 4 mg/kg bw respectively) and in adult rats (34%, 41% and 64% at 2, 4 and 6 mg/kg bw respectively). Clinical signs (salivation, spontaneous vocalization and burrowing behaviour) were evident only at the highest doses in each age group of rats. Only mild salivation was observed at 2 mg/kg bw. At the dose which induced a 50% decrease in ASR (4 mg/kg bw), the brain concentration of deltamethrin was two fold higher in weanling than in adult rats. LD_{50} determinations were made in 11, 21 and 72 day old rats. Six animals/age/dose level were treated: Groups of 11-day old pups received deltamethrin at 0, 2, 4, 8, 12 and 16 mg/kg bw, 21 day-old animals at 0, 2, 4, 6, 8, 10, 12 and 16 mg/kg bw, and adult rats at 0, 60, 80, 100, 120 and 140 mg/kg bw. The LD50 for 11, 21, and 72 day old rats were 5.1, 11, and 81 mg/kg bw respectively. Weanling and adult rats died 30-90 minutes and 2-3 minutes after treatment respectively. In neonatal (11-day old) rats the acute lethality of deltamethrin was 16-fold that in adult animals. The brain concentration of deltamethrin at lethal doses was comparable in weanlings receiving 12 mg/kg and adult rats receiving 80 mg/kg. These results demonstrate an age-dependent difference in susceptibility of male rats to an acute dose of deltamethrin. 11-day old rats and 21-day old rats were 16 and 7-fold more sensitive than adults, but brain concentration of the compound was comparable in 21-day old and adult rats. The difference is due to the relative amount of chemical that reaches the target tissue (disposition), rather than a difference in binding site characteristics (functional). The data indicate that the age-related susceptibility is attributable to the pharmacokinetics of deltamethrin rather than to a difference in susceptibility of the target tissue, the brain. In fact, the same concentration in brain was associated with the same clinical effects in both young and adult animals. A metabolic deficiency in young animals is the most likely mechanism for the age-dependent susceptibility of rats to deltamethrin. This effect is a particular characteristic of type II pyrethroids, containing a cyano group (Sheets et al., 1994).

Table 3**. Age-dependent neurotoxicity in rats**

Strain	Treatment	Test	Clinical signs	Deltamethrin content in brain (µg/g)	Reference

Long Evans weanling rats 21days old (8 pups per dam) 1 m and 1 f /12 litters	0,1, 2 and 4 mg/kg bw in corn oil (1 ml/kg) by gavage	**Behavioural test** Dose related decrease ASR (acoustic startle response) 2 h after treatment 1 mg 18 % 2 mg 39 % **4 mg 59 %** No sex difference	**Mild salivation salivation, spontaneous vocalization, burrowing behaviour** No sex difference	**0.05 ± 0.01** (0.008-0.056)	Sheets et al., 1994
Long Evans adult rats 72-day old 8 (6m 2f) per dose level	0, 2, 4 and 6 mg/kg bw	2 mg 34 % 4 mg 41 % **6 mg 64 %**	Mild salivation	**0.023 ± 0.006** (0.015-0.026)	
Long Evans non fasted rats 6 animals per age per dose		**Lethality test(LD_{50}) mg/kg bw (range)**	**Profuse salivation, coarse tremors, choreathatosis** (all groups)		Sheets et al., 1994
11days old	0,2,4,8, 12 and16 mg/kg bw	5.1 (steep dose response			
21 days old	0,2,4,6,8,10, 12 and 16 mg/kg bw	**11 (8-14) 30-90 min**		**0.129± 0.015** (0.118-0.147)	
72 days (adult)	0, 60, 80, 100, 120 and 140 mg/kg bw	**81 (59-95) 2-3 hours**		**0.145 ± 0.063** (0.068-0.217)	

2.2.3. Toxicokinetic factors

A study designed to investigate the age-dependent metabolism of deltamethrin confirmed the age-related sensitivity of rats to acute doses. Ten- and 21-day old male Sprague Dawley rats treated by gavage with 10 mg/kg bw of deltamethrin in glycerol showed clinical signs of poisoning (salivation and tremors) leading to 100% mortality, while 40- and 90-day old rats at the same dose experienced only transient salivation and survived. In vitro studies were carried out to characterize the differential efficiency in metabolizing deltamethrin by plasma and liver from 10-, 21-, 40- and 90-day old rats. In adult rats and mice, deltamethrin is metabolized by ester cleavage (plasma and hepatic esterases) and by cytochrome P450-catalyzed hydroxylation with subsequent glucuronidation and sulfation. The study showed that the capacity of plasma carboxylesterases (CaEs) and P450s to detoxify deltamethrin reaches adult levels in 40-day old animals, although the Vmax and Km were further increased in 90-day old rats. In contrast, the efficiency of liver CaEs in 40-day old rats was only 21% of that in 90-day old rats. The limited capacity of these enzymes contributes significantly to increased systemic exposure and neurotoxic effects in immature rats. The age-dependent increase in deltamethrin metabolism observed *in vitro* is consistent with the age-dependent decreases in blood deltamethrin levels and toxicity *in vivo*. Given that the parent compound is responsible for the neurotoxic effects, incomplete development of detoxifying enzymes is a plausible explanation for the age-dependent sensitivity to the compound (Anand et al., 2006).

Qualitative and quantitative interspecies differences in enzymes involved in deltamethrin biotransformation have also been observed. In humans the maturation of metabolic capacity is different: preliminary results suggest that, in contrast to rodents, human liver carboxylesterase expression is at near adult levels within three months and changes relatively little during postnatal maturation (Pope et al., 2005).

A dose-dependent increase in mRNA expression levels of both CYP1A1 and CYP1A2 and in their respective catalytic activities was observed in the brains and livers of rat offspring following prenatal exposure to low doses (0.25 or 0.5 or 1.0 mg/kg bw p.o.) of deltamethrin, of pregnant dams from gestation days 5 to 21. The consequence of these experimental observations for the neurotoxic effects of the compound is not clear. However, the persistence of the increase in mRNA expression of CYPs even through to adulthood indicated an imprinting of the CYPs as has been observed following exposure to low doses of many other CYP inducers such as benzo(a)pyrene. It has to be considered that in this study the rats were treated with Decis 2.8%, an emulsion that contains 2.8% technical grade deltamethrin together with a number of well known CYP inducers (Johri et al., 2006 a and b).

In addition, age-dependent toxicokinetic factors other than metabolism may affect the bioavailability of deltamethrin and the magnitude of its adverse effects. Oral deltamethrin lethality and neurotoxic activity are largely due to increased GI absorption and bioavailability following dissolution of the chemical. The vehicle in which deltamethrin is administered orally has a relevant influence on dissolution, absorption and bioavailability, as well as deposition in target and storage tissues. Bioavailability and target organ (brain) levels were significantly higher when the insecticide was given orally to rats in a solvent solution (as glycerol formal (GF), a binary solvent, a condensation product of glycerol and formaldehyde) than as an unstable suspension. Rats receiving 10 mg deltamethrin/kg bw per os in GF exhibited transient salivation and tremors, whereas animals treated with the same dose in a vegetable oil were asymptomatic. The processes or mechanisms by which deltamethrin and other pyrethroids are absorbed across the gastrointestinal epithelium and enter the systemic circulation are not clear. The efficiency of gastrointestinal absorption is greater from solvent-based products than from aqueous suspensions. From the data available, it can be suggested that the oral bioavailability and toxic risks posed to humans by formulations of deltamethrin will be relatively modest (Kim et al., 2007).

Preweanling rats have been shown to have relatively high gastrointestinal absorption rates: extensive absorption from the GI tract of young animals could contribute to the higher internal exposure and toxicity of deltamethrin in young rats.

2.2.4. Mode of action

While toxicokinetic differences have been demonstrated for deltamethrin between adult and developing animals, toxicodynamic factors have not been systematically investigated. The primary mode of deltamethrin's action in both insects and mammals is the reversible disruption of voltage-sensitive sodium channels' (VSSCs) activity (or function). VSSCs are crucial for control of electrical excitability and are involved in the initiation and propagation of action potentials.

In general, deltamethrin, a type II pyrethroid, holds open the channels for such long periods of time that the membrane potential ultimately becomes depolarized to the point at which generation of action potentials is not possible (depolarization-dependent block).

Mammalian VSSCs are composed of one alpha and two beta subunits. Ten different alpha subunits (Nav1.1–1.9, Nax) and four different beta subunits (β1–β4) have been identified and they are expressed in a tissue-, region- and time-specific manner. Alpha subunits form the pore of the channel and the beta subunits are localized in the membrane and interact with cytoskeletal proteins influencing the gating properties. Embryonically expressed forms of VSSCs are replaced by expression of adult forms as neurodevelopment proceeds. Expression of both α and β subunits is developmentally regulated in the nervous system, with Nav1.3 and β3 subunits predominating in the embryonic brain, while Nav1.2, Nav1.6 and β1 subunits are highly expressed in the adult central nervous system.

The complex ontogeny of VSSC expression could result in altered sensitivity (either increases or decreases) of the developing nervous system to perturbation by various pyrethroids. Perturbation of VSSC function during development impairs nervous system structure and function. Knockout and mutant mouse models of sodium channel α subunits demonstrate varying degrees of adverse outcomes associated with loss or alteration of specific channel subunits. In humans, perturbation of nervous system development has been associated with altered VSSC structure or function. Recent advances in molecular genetics have identified in genes coding for VSSC subunits a number of mutations that result in neuronal hyperexcitability due to subtle changes in channel gating and inactivation. These mutations have been linked to various forms of epilepsy in humans, providing evidence that changes in VSSC function can give rise to clinical disease. Pyrethroids, like these mutations, are known to alter VSSC activation and inactivation, and hence neuronal excitability. However, these changes are transient and associated with the reversible interaction with VSSC. At current occupational exposures and exposures via food residues, such interactions are not expected to occur in the CNS.

A very recent study has demonstrated significant differences in pyrethroid action on VSSCs expressed in *Xaenopus laevis* oocytes that are dependent upon channel subunit composition. Specifically, compounds containing an α-cyano group modify both Nav1.2 and Nav1.3 containing channels. The presence or absence of a β subunit significantly enhances the fraction of Nav1.3 channels modulated by deltamethrin (Meacham et al., 2008).

3. Conclusions

3.1. Conclusion to question 1

Question 1: Based on the available data, both in the published scientific literature and in the toxicological dossier submitted for inclusion in Annex I, does the PPR Panel consider that deltamethrin shows developmental neurotoxic effects?

Deltamethrin is a type II pyrethroid producing a syndrome of choreoathetosis and salivation in experimental animals (CS syndrome). Major signs of acute poisoning include salivation, hyperexcitability (occurrence of involuntary movements in a combination of chorea and athetosis) and clonic seizures.

Studies in adult rats revealed effects on motor activity, motor and sensory functions, and learning and memory after acute exposure to high doses of deltamethrin (50 mg/kg bw). Clinical signs peaked 3 hours after treatment and the effects were transient. Less severe effects were noted in a short term study (13-week feeding study) in rats, where at the highest concentration tested (800 ppm equal to 54 and 58 mg/kg bw/day for males and female respectively), a functional observational battery revealed changes such as gait alterations, hypersensitivity to noise and impaired righting reflex, but no treatment-related effect on locomotor activity, neuropathological lesions or changes in brain weight or dimensions were observed.

The potential developmental neurotoxicity of deltamethrin was addressed by the notifier in guideline-compliant reproductive and developmental toxicity studies in different animal species, including a multi-generation study in rats and a separate developmental neurotoxicity (DNT) study in rats complying with the new OECD guideline TG426.

Signs of neurotoxicity attributable to deltamethrin were observed in F_0 and F_1 generations of rats in a two generation study only at the highest administered dose (320 ppm equal to 18 mg/kg bw/day). Clinical signs such as ataxia and hyperactivity, vocalization, and excessive salivation occurred at this dose without any associated neuropathological lesion. Convulsions and increased salivation were the only clinical signs of maternal toxicity related to deltamethrin exposure at the highest concentration tested in developmental studies in mice, rats and rabbits, while no neurotoxic effects were detected in the offspring. The evidence from standard studies is that offspring exposed pre- or postnatally are not more sensitive than adults in the same experiment.

The new developmental neurotoxicity study (DNT) carried out in Wistar rats involved treatment of females via the diet during gestation and lactation, and the consequent exposure of offspring during prenatal and neonatal life, the sensitive period of neuronal development. The evaluation included assessment of physical development, development of reflexes, motor activity, motor and sensory function, learning and memory, and also determination of brain weight and neuropathological evaluation during postnatal development and adulthood. Although there was an absence of specific determination of deltamethrin in milk and in brain tissues in this study, evidence for lactational transfer of deltamethrin was obtained in a pilot study in which offspring brain concentrations of deltamethrin were determined. In addition, the developmental effects observed in the pups, such as decrease in body weight gain and in body weight, and delayed sexual maturation in males indicate that exposure of the pups was effective. Another effect observed in the offspring was an increased incidence of vocalizations with handling in males on PND 4. The toxicological significance of this isolated finding is not clear. The Panel concluded that no clear evidence of a neurotoxic effect was detected in this

study, and the NOAEL for offspring was 80 ppm (equal to 6.78 mg/kg bw/day) based on reduced bodyweight gain (>10%) and delayed balanopreputial separation noted in offspring at a concentration of 200 ppm (equal to 16.1 mg/kg bw/day).

The data available from the open literature include studies carried out in rodents involving exposure during prenatal life from GD 5-20 and/or during postnatal life from PND 1 to PND 20.

Three studies in mice used the same experimental design: deltamethrin was administered orally via a PVC tube as a daily dose for 7 days to 10-day-old NMRI mice, followed by evaluation of locomotor activities and effects on muscarinic receptor density in the CNS.

The first study, in which mice were killed 24 h after the last administration, revealed transient neurotoxic effects associated with a decrease (7%) in the density of muscarinic receptors in the hippocampus and an increase (10%) in the density of nicotinic receptors in the cerebral cortex after exposure to 1.2 mg/kg bw/day. Mice exposed to a lower dose (0.7 mg/kg bw/day) showed no behavioural signs, but had an increase in muscarinic receptor density (8%) and nicotinic receptor density (21%) in the cortex (Eriksson and Nordberg, 1990).

A second study, carried out in the same laboratory (Eriksson and Nordberg, 1991), used the same treatment schedule at the lower dose (0.7 mg/kg bw/day) and evaluated the persistence of any neurotoxic effects induced. In 17-day old mice, at the end of treatment, no significant change in behavioural variables was observed confirming the transitory nature of the neurotoxic clinical signs observed in adult and neonatal life. In adult (4-month-old) mice, a statistically significant increase in locomotion and total activity was observed, but only during the last 20 minutes of an observation period of 60 minutes. Although it is specified in the paper that each treatment group contained 12 mice from three different litters, no data about the interindividual variability for the behavioural tests were reported. A decrease in the density of muscarinic receptors in the cerebral cortex that was detected in the same animals was not statistically significant ($0.05 \leq p \leq 0.1$). Interpretation of the findings from these two studies might have been helped by additional investigations, but no further papers were published by the laboratory concerned. In view of the weak statistical significance and inconsistency of the findings on receptor densities, the data from this study do not clearly indicate developmental neurotoxicity.

In a further unpublished study (Muhammad and Ray, 1997) cited in the DAR and in Shafer et al. 2005, which used the same treatment schedule at a low dose (0.7 mg/kg bw/day) contrasting results were obtained. An increase of activity and a decrease in habituation were observed in adult mice exposed to deltamethrin dissolved in an emulsion (egg lecithin: peanut oil 1:10 40% in water) as was used in the experiments by Eriksson and Nordberg. An increase in receptor density (19%) was observed in 17-day old mice, but no effect on behavioural tests.

A decrease in habituation was observed in one of two experiments using deltamethrin dissolved in corn oil without any variation in the muscarinic receptor density. On the contrary a decrease in receptor density was detected in the other experiment.

Two studies are available in Wistar rats involving exposure during prenatal life. The first one, at a dose of 0.08 mg/kg bw administered daily during GD6-GD15, showed an increase of rearing at PND21 and a decrease in locomotion frequency and in immobility time in a swimming test at PND60, only in males, associated with an increase in NA, DOPAC and DOPAC/DA without changes in DA or HVA concentrations (Lazarini et al., 2001). The other study, involving treatment of the females from GD14 to GD20 at a dose of 1 mg/kg bw/day, reported an age-related neurotoxic effect characterised by clinical signs, associated with a

parallel change in receptor density at 6 and 12 weeks of age. An increase in AChE activity in the hippocampal region (28% and 16%) and a decrease in muscarinic receptors (48% and 39% at 6 and 12 weeks, respectively) were observed (Aziz et al., 2001). A study involving exposure during neonatal life from PND 9-PND 13, demonstrated a delay in the process of cytogenesis and morphogenesis of micro-neurons and inter-neurons in the cerebellum. No direct neurotoxic effects were investigated (Patro et al., 1997)

More information about the age-dependent difference in susceptibility of rats to deltamethrin comes from the study of Sheets at al. (1994) involving the treatment of separate groups of weanling and adult mice with behaviourally active and lethal doses of the compound. In weanling rats the acute lethality of deltamethrin was 7-fold greater than in adult rats, with comparable brain concentrations of the compound, indicating that the age dependency was due to a difference in the disposition of the compound rather than in the binding site characteristics. In addition age-dependent differences in sensitivity to deltamethrin were apparent only at lethal doses. At the low, behaviourally active dose, developing animals seemed less sensitive than adults: the same dose (4 mg/kg bw) was able to reduce the amplitude of the acoustic startle response (ASR) by approximately 50% (EC_{50}) in adults and in weanling rats, although an approximately two-fold higher brain concentration of the compound was detected in weanling rats.

A number of experimental studies from the open literature reported effects at relatively low doses. However, the PPR panel noted that there is a lack of consistency in these effects, which were sometime contradictory both within and between studies and there was often equivocal statistical significance. The panel concluded that these effects were not a secure basis on which to conclude that deltamethrin is a developmental neurotoxicant.

The PPR Panel concludes that the available data do not indicate that deltamethrin is a developmental neurotoxic agent.

3.2. Conclusion to question 2

Question 2: Has deltamethrin been assessed adequately for developmental neurotoxicity and if not what further information would be of value in this assessment?

The potential developmental neurotoxicity of deltamethrin was addressed by the notifier in guideline-compliant reproductive and developmental toxicity studies in different animal species including a multi-generation study in rats and a separate DNT study in rats according to the new OECD guideline TG426.

The rat was the chosen species, as recommended by the guidelines. Furthermore, from the neurological findings in short-term toxicity studies, the rat appeared to be the most sensitive species to these effects.

Each test and control group contained a sufficient number of pregnant females (30/group) to ensure that an adequate number of offspring was produced for neurotoxicity testing. The animals were exposed during prenatal life *in utero*, then through lactation and through direct consumption of food during neonatal life. The treatment period included the critical stage of neonatal brain development in rodents when exposure to a neurotoxic agent can lead to irreversible changes in adult brain function, and also to an increased susceptibility to toxic agents at adult ages.

Deltamethrin technical grade was administered to mated female rats via the diet, the route most relevant to potential human exposure. Three dose levels 0, 20, 80 and 200 ppm (equal to 0,

1.64, 6.78 and 16.1 mg/kg bw/day) were used. Concurrent negative controls were included. Effects on the dams' body weight (7% decrease) and body weight gain (17% decrease) at the highest dose suggest that a Maximum Tolerable Dose (MTD) was reached.

Although specific determination of deltamethrin in milk and in brain tissues was not undertaken in this study, evidence was obtained in the pilot study for lactational transfer of deltamethrin, which was found in brain tissues at Post-Natal Day (PND) 10, PND14, and PND16. Given the rapid elimination of deltamethrin, this supports the assumption that pups were exposed to the compound via milk. In addition, the occurrence of systemic toxicity in pups, such as persistent reductions in body weight gain and in body weight and delayed sexual maturation in males, provides evidence that the offspring were continuously exposed. This evidence is further supported by pharmacokinetic studies showing that deltamethrin is excreted via the milk in lactating dairy cows.

The offspring (16 animals/sex) were evaluated by detailed clinical observations, and assessment of body weight, food consumption, developmental landmarks for sexual maturation, automated measures of activity, auditory startle habituation, learning and memory, and an ophthalmic examination. Tissues were collected for morphometry (brain) and microscopic examination on PND21 (brain) and at study termination (brain, an assortment of additional neural tissues, and skeletal muscle).

The PPR Panel concludes that deltamethrin has been adequately tested for developmental neurotoxicity.

3.3. Conclusion to question 3

Question 3: Do the existing health-based guidance values provide adequate protection against any potential developmental neurotoxicity of deltamethrin, and if not, what values would be necessary to provide such protection?

Table 4 summarizes all of the available studies evaluated for the purpose of risk assessment.

The existing health-based guidance values for deltamethrin are based on neurological signs as the most relevant critical effects observed in adult animals from different species.

The lowest NOAEL of 1 mg/kg bw/day, used for risk assessment, was obtained in the 90-day and 1-year dog studies. Based on this NOAEL with a 100-fold safety factor (SF) (standard safety factor) the guidance values are:

ADI = NOAEL/SF = 1 mg/kg bw/day/100 = 0.01 mg/kg bw/day
ARfD = NOAEL/SF = 1 mg/kg bw/100 = 0.01 mg/kg bw
AOEL = NOAEL/SF = 1 mg/kg bw/day/100 x 0.75 (adjustment for gastrointestinal absorption) = 0.0075 mg/kg bw/day
The study performed according to OECD guideline 426 provides state-of-the-science evaluation of the potential for developmental neurotoxicity and in this study no developmental neurotoxicity was observed at the highest dose tested of 80 ppm (equal to 6.78 mg/kg bw/day). This gives a margin of safety of more than 600, relative to the existing guidance values.

The PPR panel concludes that the existing health-based guidance values provide adequate protection against any potential developmental neurotoxicity of deltamethrin, that in any case would occur only at doses causing severe systemic toxicity.

Table 4 **Studies evaluated for risk assessment**

Species	Study Treatment	Effects	NOAEL mg/kg bw/day	LOAEL mg/kg bw/day
Short-term studies				
Mice (Swiss) 10 animals/sex/group **Fabreguettes, 1991**	12-week dietary study (0, 6, 62, 603, 1318 m; 0, 8, 77, 739, 1391 f mg/kg bw/day)	Mortality neurological signs, poor condition, reduced bw, bw gain and food consumption	Males: No NOAEL Females: 77	6: reduced bw gain 739: mortality (1/10) reduced bw gain and food consumption
Rats (Sprague Dawley) 20 animals/sex/group **Ryle et al., 1991a**	13-week dietary study (2, 24, 72, 241, 425 m; 3, 30,84, 272,444 f mg/kg bw/day)	Mortality, neurological signs, reduced bw and bw gain and food consumption	Males: 24 Females: No NOAEL	72: death, neurological disturbances 3: reduced bw gain
Rats (Sprague Dawley) 20 animals/sex/group **Hunter, 1977**	13-week oral gavage 0, 0.1, 1.0, 2.5, 10 mg/kg bw/day in PEG200 Recovery 4 weeks	Hypersensitivity, reduced bw gain	Males: 1 Females: 2.5	2.5: reduced bw gain 10: hypersensitivity
Dogs (Beagle) 3 animals/sex/controls and 0.1mg group 5 animals/sex/ 1, 2.5, and 10 mg groups **Chesterman, 1977**	13-week oral by gelatine capsule in PEG200 (0, 0.1, 1.0, 2.5, 10 mg/kg bw/ day)	Neurological signs (reversed following cessation of dosing), liquid faeces, dilatation of pupils decreased bw gain and food consumption	Males and females: 1	2.5: liquid faeces and dilatation of pupils, body tremor
Dogs (Beagle) 6 animals/ sex/controls and 50 mg group 3 animals/sex/, 2, and 10 mg groups) **Ryle et al., 1991b**	13-week oral by gelatine capsule in PEG200 (0, 2, 10, and 50 mg/kg bw/ day)	Neurological signs (tremors) salivation, reduced bw and food consumption	Males and females: 10	50: neurological effects, reduced bw and food consumption)
Long-term studies				
Mice (Charles River CD-1) 80 animals/sex/group **Goldenthal, 1980a**	24 month-dietary study (0, 0.12, 0.6, 3.0, 12 m 0, 0.15, 0.75, 3.8, 15 f mg/kg bw/day) in corn oil	Reduced bw gain, organ weight	Males: 12 Females: 15	No effect at any dose level

Mice (CD-1) 50 animals/sex/group **Richard, 1995**	97 week-dietary study (0, 2, 16, 155, and 315 m; 0, 2, 20, 189 and 395 f mg/kg bw/day)	Clinical signs (dyspnoea, emaciation) skin lesions, reduced bw gain	Males: 16 Females: 189	155: (skin lesions) 395: (skin lesions,dyspnea, emaciation)
Rats (Charles River CD) 90 animals/sex/group **Goldenthal, 1980b**	24-month-dietary study (0, 0.1, 0.8, 2,1 m; 0, 0.1, 1.1, 2.8 f mg/kg bw/day)	Increased mean weight of uterus, adrenals, thyroid, pituitary in females and testis in males. Decrease of mean thyroid weight in males	Male: 0.8 Females: 1.1	2.1: decreased bw and variation of mean weight of various organs
Rats (Crl:CD(SD)BR) 70 animals/sex/group **Ryle, 1995**	104-week dietary study (0, 1, 5, 22, 36 m; 0, 2, 7, 30, 47 f mg/kg bw/day)	Incoordinated movements of limbs, decreased bw, increased plasma glucose, decreased white blood counts, plasma cholesterol and albumin	Males: 1 Females: 30	Hepatotoxicity increased ballooned cells, neurological signs
Dogs (Beagle) 4 animals/sex/group **Ryle et al., 1993**	One-year oral by gelatine capsule in PEG200 (0, 1, 10, and 50 mg/kg bw/day)	Neurological signs, salivation, reduced bw and food consumption, decreases in serum albumin and calcium concentrations	Males and females: 1	10: behavioural changes and liquid faeces
Reproductive toxicity studies				
Rats (Crl:CD BR VAF/Plus) 30 animals/sex/group **Hoberman, 1992**	Two generation study 0, 5, 20, 80, or 320 ppm	Gastric erosions, clinical signs (hypersensitivity ataxia, impaired righting reflex, hyperactivity, splayed limbs) reduced bw and food consumption, increased mortality	Adults: 4.2 Offspring: 11	18: clinical signs in females during gestation and lactation, reduced bw gain and food consumption, and increased mortality 18: reduced body weight, clinical signs, reduced viability, and increased mortality rates before and after weaning up to 18 days.

Mice(CD1) 30 animals /group **Kavlock et al., 1979a**	Developmental study 0, 3, 6, or 12 mg/kg bw on days 7–16 of gestation . Mice killed on day 18 of gestation in corn oil	Reduced maternal bw gain and convulsions Increase in the occurrence of supernumerary ribs in neonates	Maternal: 3 Neonatal: no NOAEL	6: reduced body weight and convulsions increased supernumerary ribs in all the treated groups
Rats (Sprague-Dawley) 30 animals /group **Kavlock et al., 1979b**	Developmental study 0, 1.2, 2.5, or 5.0 mg/kg bw on days 7–20 of gestation. Rats killed on day 21 of gestation in corn oil.	Reduced bw gain and salivation	Maternal:2.5 Neonatal : 5	5: reduced bw gain and mild salivation Absence of malformations or other effects at the highest dose
Rats (Charles River Crl :CD VAF/Plus) 25 animals /group **Schardein, 1990**	Developmental study 0, 1, 3.3, 7, 11 mg/kg bw/day on days 6–15 of gestation in corn oil.	Clinical signs (moribundity, convulsions, increased salivation, hypersensitivity, staining), reduced body weight	Maternal toxicity: 3.3 Neonatal toxicity: 11	11: clinical signs and reduced bw Absence of malformations or other effects at the highest dose
Rabbit (New Zealand White) 24 animals/dose **Richard, 2001**	Developmental study 0, 3, 10 or 32 mg/kg bw/day from day 6 to day 28 *post-coitum* inclusive.	Reduced bw gain and food consumption	Maternal toxicity: 10 Neonatal toxicity: >32	32: reduced bw gain and food consumption Absence of malformations or other effects at the highest dose
Neurotoxicity studies				
Rats (Sprague-Dawley) 12 animals/sex/group **Nemec, 1998a**	Acute neurotoxicity study. Single dose oral gavage 0, 5, 15, or 50 mg/kg bw in corn oil "functional observation battery" and locomotor activity before treatment and 3 h, 7 days, and 14 days later. The rats were killed on day 15. Neuropathological examination of central and peripheral neural system tissues.	Functional domains affected:. Sensorimotor, autonomic, neuromuscular, physiological, activity, and excitability decreased mean body weight gain	Males and females: 5	15: functional observation battery and locomotor activity

Rats (Sprague-Dawley) 10 animals/sex/group **Nemec, 1998b**	13-week dietary study 0, 4, 14 and 54 mg/kg bw/day for males, and 4, 16 and 58 f mg/kg bw/day for females, for 13 weeks "functional observation battery" and locomotor activity before treatment and during weeks 4, 8, and 13 after treatment.	Piloerection and slightly soiled fur in the home cage; impaired mobility and gait and bizarre or stereotypic behaviour (rocking side-to-side) in the open field; altered air righting reflex; and altered hindlimb extensor strength and reduced forelimb and hindlimb strength. decrease mean body weight gain and food consumption	Males and females: 4	14: hypersensitivity to noise and gait alterations
Rats (Wistar) 30 animals/sex/group **Gilmore, 2006**	Developmental neurotoxicity dietary study 0, 1.64, 6.78 and 16.1 mg/kg bw/ day from GD 6 to LD 21 Clinical observations, "functional observational battery", automated measures of activity, auditory startle habituation, learning and memory, ophthalmic examination. Neural tissues (on PND 21 and at study termination 75 days of age) for microscopic examination and morphometry	Decreased bw, bw gain and food consumption Increased vocalization in male at PD4, delayed onset of balanopreputial separation	Maternal: 6.78 Offspring: 6.78	16.1: decreased bw, bw gain and food consumption 16.1:decreased bw, bw gain and food consumption. Increased vocalization in male at PD4, delayed onset of balanopreputial separation.

CONCLUSIONS AND RECOMMENDATIONS

Question 1

The PPR Panel re-evaluated the available data in the published literature, in the toxicological dossier and other relevant information to assess the possible developmental neurotoxicity of deltamethrin. Results from standard studies indicate that offspring exposed pre- or postnatally are not more sensitive than adults in the same experiment. No evidence of neurotoxic effects

was detected in a new developmental neurotoxicity study carried out in Wistar rats, involving exposure of offspring during prenatal and neonatal life, the sensitive period for neuronal development. The results from the open literature indicate that the age-dependent variation in the toxicity of deltamethrin is apparent mainly at high acute doses, and is a consequence of toxicokinetic rather than toxicodynamic differences.

The PPR Panel concludes that the available data do not indicate that deltamethrin is a developmental neurotoxic agent.

Question 2

The potential developmental neurotoxicity of deltamethrin was addressed by the notifier in guideline-compliant reproductive and developmental toxicity studies in different animal species, including a multi-generation study in rats and a separate developmental neurotoxicity (DNT) study in rats complying with the new OECD guideline TG426.

The PPR Panel concludes that deltamethrin has been adequately tested for developmental neurotoxicity.

Question 3

The actual guidance values are:

ADI = NOAEL/SF = 1 mg/kg bw/100 = 0.01 mg/kg bw/day

ARfD = NOAEL/SF = 1 mg/kg bw/100 = 0.01 mg/kg bw

AOEL = NOAEL/SF = 1 mg/kg bw/day/100 x 0.75 (adjustment for gastrointestinal absorption) = 0.0075 mg/kg bw/day

The study according to OECD DNT guidelines provides a state-of-the-science evaluation of the potential for developmental neurotoxicity, and in this study no developmental neurotoxicity was observed at the highest dose tested, 80 ppm (equal to 6.78 mg/kg bw/day). This provides a margin of safety of at least 600 relative to the existing guidance values (ADI = 0-0.01 mg/kg bw/day, ARfD = 0.01 mg/kg bw) for any potential developmental neurotoxic effect.

The PPR panel concludes that the existing health-based guidance values provide adequate protection against any potential developmental neurotoxicity of deltamethrin that would in any case occur only at doses causing severe systemic toxicity.

DOCUMENTATION PROVIDED TO EFSA

1. Documents from RMS
   - Addendum to the DAR for deltamethrin – Annex B. Section 5. Toxicology – rev. 2 July 2002
   - New Annex II data post Annex I inclusion – B.6. Mammalian toxicology – Sweden (September 2007)
   - Swedish position deltamethrin, - re-evaluation of guidance values (10 January 2008)

- Swedish position paper on the neurotoxicity of deltamethrin (9 May 2008)

2. Comments of other Member States on the Swedish position (January-February 2008)
   - BE comments
   - BG comments
   - DE comments
   - DK comments
   - ES comments
   - FI comments
   - IT comments
   - IR comments
   - LV comments
   - NL comments
   - UK comments
3. Notifier's comments
   - Letter and Position paper BCS of 29 January 2008
   - Letter BCS on US-EPA data evaluation record for the deltamethrin developmental neurotoxicity study (9 May 2008)
4. Other documents
   - US-EPA data evaluation record on the DNT study conducted with deltamethrin (18 December 2007)

REFERENCES

Anand SS, Kim KB, Padilla S, Muralidhara S, Kim HJ, Fisher JW and Bruckner JV, 2006. Ontogeny of hepatic and plasma metabolism of deltamethrin *in vitro*: role in age-dependent acute neurotoxicity. Drug Metab. Dispos. 34, 389-397.

Aziz MH, Agrawal AK, Adhami VM, Shukla Y, Prahlad Kishore Seth PK, 2001. Neurodevelopmental consequences of gestational exposure (GD14-GD20) to low dose deltamethrin in rats. Neurosci. Lett. 300, 161-165.

Eriksson P and Nordberg A, 1990. Effects of two pyrethroids, bioallethrin and deltamethrin on subpopulations of muscarinic and nicotinic receptors in the neonatal mouse brain. Toxicol. Appl. Pharmacol. 102, 456-463.

Eriksson P and Fredriksson A, 1991. Neurotoxic effects of two different pyrethroids, bioallethrin and deltamethrin, on immature and adult mice: changes in behavioural and muscarinic receptor variables. Toxicol. Appl. Pharmacol. 108, 78-85.

Husain R, Malaviya M, Seth PK and Husain R, 1994. Effect of deltamethrin on regional brain polyamines and behaviour in young rats. Pharmacol. Toxicol. 74, 211-215.

Johri A, Dhawan A, Singh RL and Parmar D, 2006a. Effect of prenatal exposure of deltamethrin on the ontogeny of xenobiotic metabolizing cytochrome P450s in the brain and liver of offspring. Toxicol. Appl. Pharmacol. 214, 279–289.

Johri A, Yadav S, Singh RL, Dhawan A, Ali M and Parmar D, 2006b. Long lasting effects of prenatal exposure to deltamethrin on cerebral and hepatic cytochrome P450s and behavioral activity in rat offspring. Eur. J Toxicol 544, 58–68.

Kim KB, Anand SS, Muralidhara S, Kim HJ and Brucker JV, 2007. Formulation-dependent toxicokinetics explains differences in the GI absorption, bioavailability and acute neurotoxicity of deltamethrin in rats. Toxicology 234, 194–202.

Lazarini CA, Florio JC, Lemonica IP and Bernardi MM, 2001. Effects of prenatal exposure to deltamethrin on forced swimming behavior, motor activity, and striatal dopamine levels in male and female rats. Neurotoxicol. Teratol. 23, 665-673.

Makris SL, Raffaele K, Allen S, Bowers WJ, Hass U, Alleva E, Calamandrei G, Sheets L, Amcoff P, Delrue N and Crofton KM, 2008. A retrospective performance assessment of the developmental neurotoxicity study in support of OECD Test Guideline 426. Environ. Health Perspect. available online at http://dx.doi.org/, 12 August 2008.

Meacham CA, Brodfuehrer PD, Watkins JA and Shafer TJ, 2008. Developmentally-regulated sodium channel subunits are differentially sensitive to α-cyano containing pyrethroids. Toxicol. Appl. Pharmacol. 231, 273-281.

OECD (Organisation for Economic Co-operation and Development), 2007.Guidelines for the testing of chemicals No. 426. Developmental Neurotoxicity.

Patro N, Mishra SK, Chattopadhyay M and Patro IK, 1997. Neurotoxicological effects of deltamethrin on the postnatal development of cerebellum of rat. J. Biosci. 22, 117–130.

Pope CN, Karanth S, Liu J and Yan B, 2005. Comparative carboxylesterase activities in infant and adult liver and their *in vitro* sensitivity to chlorpyrifos oxon. Regul. Toxicol. Pharmacol. 42, 64–69.

Shafer TJ, Meyer DA and Crofton KM, 2005. Developmental neurotoxicity of pyrethroid insecticides: critical review and future research needs. Environ. Health Perspect. 113, 123-136.

Sheets LP, Doherty JD, Law MW, Reiter LW and Crofton KM 1994. Age-dependent differences in the susceptibility of rats to Deltamethrin. Toxicol. Appl. Pharmacol. 126, 186-190.

ABBREVIATIONS

5-HT	serotonin
5-HIAA	5-hydroxyindolacetic acid
AChE	acetylcholinesterase
ADI	Acceptable Daily Intake
AOEL	Acceptable Operator Exposure Level

ARfD	Acute Reference Dose
ASR	acoustic startle response
CaEs	carboxylesterases
CYP	cytochrome P-450
DA	dopamine
DAR	Draft Assessment Report
DOPAC	3,4-dihydroxyphenylacetic acid
DNT	developmental neurotoxicity
EC	emulsified concentrate
EC_{50}	effective concentration 50%
FOB	functional observational battery
F_0 generation	initial parent generation
F_1 generation	first filial generation
F_2 generation	second filial generation
GAP-43	growth-associated protein 43
GD	gestation day
GF	glycerol formal
GI	gastro intestinal
HVA	homovanillic acid
K*m*	Michaelis-Menten constant
LD	lactation day
LD_{50}	lethal dose 50%
MAO	monoamine oxidase
MTD	maximum tolerable dose
NA	noradrenaline
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
PEG	polyethylene glycol
PND	post natal day
SF	safety factor
Vmax	maximum initial velocity at which an enzyme catalyses a reaction
VSSC	voltage-sensitive sodium channels

GLOSSARY

acoustic startle response (ASR)

a transient motor response to an unexpected, intensive acoustic stimulus

choreoathetosis

a nervous disturbance marked by the involuntary purposeless and uncontrollable movements characteristic of chorea and athetosis

digestion chambers

vacuolated structures containing remnants of axonal and myelin material as a sign of ongoing demyelination in dorsal roots and sciatic nerves

K_m - Michaelis-Menten constant

the substrate concentration required for an enzyme to reach one-half its maximum velocity