Document Title

Novel CLP R2 classification proposal by EFSA for beta-cyfluthrin

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Executive Summary

In the 2018 EFSA publication 'Peer review of the pesticide risk assessment of the active substance beta-cyfluthrin', Section 2 reports the mammalian toxicity conclusion. On page 10/38, this conclusion includes a novel hazard classification category for beta-cyfluthrin (CLP R2 H361d) in contrast to its current non-classification for this category. The conclusion states the following:

> 'Based on the increased incidences of microphthalmia in the rat studies by inhalation, the classification Reproductive toxicant category 2 H361d Suspected of damaging the unborn child6 was proposed by the majority of the experts, excluding the RMS (see experts' consultation 2.76 in EFSA, 2018b).'

This conclusion arose for the first time in effectively the last technical review of the multi-year Annex I renewal process; despite the absence of any new data and against the recommendation of the RMS. Therefore this proposal was not known to the Applicant until the EFSA peer review publication was shared for their sanitisation and at which point it was no longer possible for the Applicant to discuss the significant change with EFSA or the RMS. The Applicant is concerned that the late notification of this significant proposal has compromised their ability to discuss it and possibly challenge it.

The Applicant asks for the Commission during their review of the EFSA opinion, to consider two aspects:

1. the novel appearance of this impactful proposal at such a late stage in the Annex I renewal process with no opportunity for a response by the Applicant

2. the RMS conclusion that **CLP Category R criteria are not met**. The scientific data have been summarised by the Applicant below and they show that the main basis for this proposal i.e. the 'increased incidences of microphthalmia in the rat studies by inhalation' is a secondary consequence following maternal toxicity, specific to a mode of action caused only by the inhalation route of exposure and due to the susceptibility of the particular animal strain used in the test laboratory. This conclusion has been previously established as it is supported by relevant studies using a different exposure route as well as an established understanding that these effects which are specific to rodent are non-human relevant. Further technical details are provided below.

CLP Category R criteria are not met for beta-cyfluthrin – a technical evaluation.

Summary

The route of exposure used in different developmental toxicity studies in rats can lead to different developmental toxicity outcomes for the same test material. In the case of cyfluthrin (the relevant read-across test material to assess beta-cyfluthrin), this difference is highlighted by the incidences of the eye malformation microphthalmia. Exposure of rats to cyfluthrin via the oral route did not affect the incidence of microphthalmia, but in a different study via inhalation, an increase was observed. Further studies confirmed that this change was a secondary consequence of inhalation-triggered maternal toxicity e.g. bradypnea, leading to reduced oxygen supply, hypoxia and hypoxemia.

Introduction

In this evaluation, reasons for the differences of results of a developmental toxicity study with exposure to cyfluthrin via inhalation and developmental toxicity studies with oral administration were examined based on comparison of systemic levels after oral and inhalation exposure and on mechanistic investigations regarding inhalation exposure of pyrethroids with consequences on oxygen supply leading to hypoxia and hypoxemia.

In a developmental toxicity study with exposure to cyfluthrin via inhalation increased incidences of unspecific malformations, including microphthalmia were observed which were not seen in two developmental toxicity studies with oral administration. Based on mechanistic investigations, this toxicity was shown to be secondary to effects of inhalation exposure on the dams in form of maternal hypoxia and hypoxemia. It is known that inhalation exposure of pyrethroids, like cyfluthrin, can cause bradypnea with consequences on oxygen supply leading to hypoxia and hypoxemia. It is proposed that the unspecific malformations, including microphthalmia, are secondary to this maternal hypoxia. If it would be assumed that the malformations were caused by treatment with cyfluthrin a relationship to the systemic dose would have to be assumed. However, a comparison of systemically available cyfluthrin levels after oral and inhalation exposure demonstrated that the systemic cyfluthrin concentrations after oral administration were much higher than after inhalation exposure, although after oral administration no unspecific malformations or increased incidences of microphthalmia occurred. Thus, it is concluded that the inhalation exposure alone led to the observed incidences of unspecific malformations, including microphthalmia. In addition, this effect of inhalation is known to be a rodent-specific phenomenon, hence associated effects are considered non-relevant to humans.
Therefore, it can be concluded that ‘the increased incidences of microphthalmia in the rat studies by inhalation’ were a secondary consequence of effects of the route of exposure (eg hypoxia and bradypnea) which are known to be non-human relevant. Hence the CLP Category R criteria are not met and no classification is warranted.

Developmental toxicity studies

Two oral developmental toxicity studies were conducted.
In one study oral doses of 0, 3, 10 and 40 mg/kg bw/day from GD 6 through 15 were administered by gavage in aqueous 1% cremophor/water as vehicle. No dose-related and thus no treatment-related incidences of malformations were noted ([redacted] 1996).
In another study, oral doses of 0, 3, 10 and 30 mg/kg bw/day from GD 6 through 15 were administered by gavage in 5 mL/kg bw lutrol 1% cremophor/water as vehicle. No dose-related and thus no treatment-related incidences of malformations were noted ([redacted], 1982).

In a developmental toxicity study in inseminated female rats with nose/head exposure for 6 hours/day on gestation days 6 through 15 to aerosol containing analytical concentrations of 0.0, 1.1, 4.7, or 23.7 mg/m^3 (experiment 1), and 0.0, 0.09, 0.25, 0.59, or 4.16 (30% O_2) mg/m^3 (experiment 2), with 90% of aerosol < 5 µm, minor maternal clinical signs and slight weight decreases at 4.16 to 23.7 mg/m^3, dose-related, significant decrease in fetal weight at 1.1 to 23.7 mg/m^3 and an increase in microphthalmia and anophthalmia at 23.7 mg/m^3 were seen, none in experiment 2 ([redacted], 1988).
In another developmental toxicity study in inseminated female rats with nose/head exposure for 6 hours/day on gestation days 6 through 15 to aerosol containing analytical concentrations of 0.0, 0.46, 2.55, 11.9 or 12.8 (+O_2) mg/m^3, maternal clinical signs were seen from 2.55 mg/m^3 on and an increased incidence of microphthalmia at 11.9 and 12.8 mg/m^3 were seen ([redacted], 1993).

These mechanistic data indicate that incidence of microphthalmia is plausibly linked to reduced maternal oxygen levels and can be considered as a non-specific secondary effect. These data are further supported by the lack of microphthalmia observed in the rat multi-generation study (Eigenberg, 1996) and none in the rabbit developmental toxicity study (Becker & Biedermann, 1992)

Table 1: Fetal (Litter) Incidence (%) of Microphthalmia for Rats exposed to Cyfluthrin ([redacted], 1993)

Fetal (Litter) Incidence (%) of Microphthalmia for Rats exposed to Cyfluthrin via inhalation				
Vehicle Control	0.46 mg/m3 air	2.55 mg/m3 air	11.9 mg/m3 air	12.8 mg/m3 air +39% Oxygen
0.8 (9.1)	**0.4 (4.3)**	**1.0 (8.7)**	**5.4 (34.8)**	**2.9 (21.7)**

Systemic exposure studies

In order to find out whether there are differences in the blood concentrations after oral and inhalation administration, the available ADME (Absorption Distribution Metabolism Excretion) studies and blood concentration measurements after inhalation exposure were used.
The following table gives an overview of the oral and inhalation doses and the related blood concentrations.

Table 2: Blood concentrations after oral and inhalation administration of cyfluthrin

Administered doses	**Blood concentrations**	**Remark**	**Reference**
Single oral administration (mg/kg bw)			
10 (vehicle: cremophor)	Max.: 0.304 µg/mL (1 hour p. admin.)	Max. oral dose in developmental toxicity studies: 40 mg/kg bw with vehicle 1% cremophor	[redacted], 1982
10 (vehicle: PEG 400)	Max.: 0.075 µg/mL		
20 (vehicle: corn oil)	Cmax: 0.385 µg/mL		Rodriguez et al, 2018
Inhalation administration (mg/m³)			
1.1	No data		[redacted], 1988
4.7	No data		
23.7	No data	Increased incidences of microphthalmia	
0.5	Not detectable		[redacted], 1996
2.5	Not detectable		
11.9	19.0 ± 13.3 pmol/mL 0.0082 ng/mL*	Increased incidences of microphthalmia	
12.8 (+ oxygen)	14.7 ± 4.4 pmol/mL 0.0064 ng/mL*	Increased incidences of microphthalmia	

* calculated with MG of 434.3 g/mol

It can be seen that blood concentrations after oral administration of 10 mg/kg bw by gavage with vehicle 1% cremophor in water were maximum 0.304 µg/mL (approx. 1 hour after one single oral administration). Much lower concentrations of maximum 0.075 µg/mL were found after oral gavage of 10 mg/kg bw in PEG400 as vehicle. A similar value of 0.385 µg/mL after 20 mg/kg bw in corn oil as vehicle was found in a publication (Rodriguez et al, 2018). The first oral developmental toxicity study was performed with 1% cremophor/water as vehicle, so this value is regarded as representative for comparison.

Since the highest oral dose in the oral developmental toxicity study with 1% cremophor was 40 mg/kg bw, the blood concentrations can be assumed to be higher than the aforementioned values for a dose of 10 mg/kg bw.
After inhalation administration of 11.9 mg/m³ plasma levels of 19.0 pmol/mL cyfluthrin were determined on day 8 of treatment (gestation day13). Although the measured blood concentrations were the result of a

repeated 8-day administration, the levels were much lower than the ones after single oral administration. Since in the inhalation developmental toxicity studies microphthalmia was seen at 11.9/12.8 mg/m^3, this blood level of 19.0 pmol/mL (0.0082 ng/mL) can be compared with the blood concentration of 0.304 µg/mL after oral administration which results in an obviously much higher blood concentration after oral administration as compared with inhalation administration.
Therefore, it can be concluded that the increased microphthalmia incidences cannot be the result of cyfluthrin treatment since due to the higher blood concentrations after oral administration they should have occurred in the oral developmental toxicity studies also. Since this was not the case, it appears that the inhalation exposure procedure could be the cause of the malformations, i.e. the bradypnea and subsequent hypoxia and hypoxemia.

Conclusion

It was evaluated why increased incidences of unspecific malformations, including microphthalmia occurred in developmental toxicity studies with exposure to cyfluthrin via inhalation but not in two developmental toxicity studies with oral administration. Based on mechanistic work, this phenomenon could be explained by an effect of the inhalation exposure on the dams in form of maternal hypoxia and hypoxemia. It is known that inhalation exposure to pyrethroids, like cyfluthrin, can cause bradypnea with consequences on oxygen supply leading to hypoxia and hypoxemia. It is proposed that the unspecific malformations, including microphthalmia, are secondary to this maternal hypoxia.
Furthermore, a comparison of systemically available cyfluthrin levels after oral and inhalation exposure in this paper demonstrated that the systemic cyfluthrin concentrations after oral administration were much higher than after inhalation exposure, although after oral administration no unspecific malformations or increased incidences of microphthalmia occurred.
Thus, it is concluded that the inhalation exposure alone led to the observed incidences of unspecific malformations, including microphthalmia.

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