

CYHALOTHRIN (addendum)

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1. EXPLANATION

Cyhalothrin is a type II pyrethroid insecticide and acaricide that is used predominantly on cattle and sheep, and to a lesser extent on pigs and goats, for the control of a broad range of ectoparasites.

The Committee evaluated cyhalothrin at its fifty-fourth meeting (Annex 1, reference 146), when it established a temporary acceptable daily intake (ADI) of 0–0.002 mg/kg bw by applying a safety factor of 500 to the lowest-observed-effect level (LOEL) of 1 mg/kg bw per day for induction of liquid faeces in dogs in a 26-week study. The high safety factor was used to compensate for the absence of a no-observed-effect level (NOEL) in this study. The ADI was designated as temporary because the Committee was concerned that neurobehavioural effects had not been adequately investigated. In order to enable a full ADI to be established, the Committee at its fifty-fourth meeting required the results of studies appropriate for identifying a NOEL for neurobehavioural effects in laboratory animals, to be submitted for evaluation in 2002.

The Committee reconsidered the toxicological data on cyhalothrin at its fifty-eighth meeting in 2002 (Annex 1, reference 157) and decided to extend the temporary ADI while awaiting the results of a study of neurobehaviour. These data were required for evaluation in 2004.

2. BIOLOGICAL DATA: TOXICOLOGICAL STUDIES

A search of the scientific literature was performed, looking for toxicological studies on cyhalothrin that had been published since 2000. It revealed several studies with lambda-cyhalothrin (λ -cyhalothrin) and one study with cyhalothrin.

Two reports of tests for genotoxicity were considered to be relevant to the evaluation of the safety of cyhalothrin. One study found that λ -cyhalothrin could cause the formation of micronuclei in the erythrocytes of exposed fish (Cavas & Ergene-Gozukara, 2003). The assay for micronucleus formation in fish is not validated for assessing genotoxic hazard to consumers, but it is sometimes used to determine whether environmental chemicals have genotoxic potential. Previously, the fifty-

fourth meeting had discounted positive results from an earlier test for micronucleus formation in fish because the assay was not validated for use in human risk assessment. The result of the new test was discounted for the same reason.

The other study investigated the effects of intraperitoneal injections of cyhalothrin on bone-marrow cells in rats (Celik et al., 2003). It showed that λ -cyhalothrin could cause the formation of micronuclei and structural chromosomal aberrations. The protocol of the study did not conform to the recommendations given in OECD guidelines 474 (assay for micronucleus formation) or 475 (assay for cytogenetic effects). Group sizes were too small, no scientific justification was given for using an extended dosing regime (seven doses given over 13 days), and bone marrow was harvested at a time after treatment (30h) that was later than recommended (18–24h for micronucleus formation or 18–27h for cytogenetic effects). The results of these mutagenicity studies contrasted with the generally negative results that had previously been obtained for cyhalothrin and for λ -cyhalothrin. The negative results of a test for cytogenetic aberrations in vivo with cyhalothrin and the negative results of a test for micronucleus formation in bone-marrow cells in mice in vivo with λ -cyhalothrin (which had already been considered by the Committee at its fifty-fourth meeting (Annex 1, references 146, 147), although these tests were performed in 1983, were well-conducted and consistent with OECD guidelines 475 and 474, respectively. The present Committee regarded the negative results from these studies to be more reliable than the positive result from the new study.

The Committee considered these new results investigating genotoxicity with λ -cyhalothrin together with the results of another mutagenicity study with cyhalothrin and λ -cyhalothrin, in order to evaluate whether residues of cyhalothrin may be genotoxic. The present Committee concluded that, when viewed as a whole, the data indicated an absence of genotoxicity for cyhalothrin and λ -cyhalothrin.

The other studies with λ -cyhalothrin were considered to be of limited relevance to the evaluation of an ADI for cyhalothrin for use in veterinary medicines, as λ -cyhalothrin is more toxic than cyhalothrin. Note that the isomer ratio of λ -cyhalothrin is different to that of the form of cyhalothrin used in veterinary medicines; λ -cyhalothrin consists entirely of the B pair of enantiomers ($Z(1R)$ *cis* (S) α -CN and $Z(1S)$ *cis* (R) α -CN), whereas cyhalothrin also contains the A pair of enantiomers ($Z(1R)$ *cis* (R) α -CN and $Z(1S)$ *cis* (S) α -CN). According to the results of studies of acute toxicity and of repeated doses, λ -cyhalothrin is more toxic than cyhalothrin and has a higher insecticidal potency (IPCS, 1990). Cyhalothrin contains the A and B pairs in a ratio of 60:40, and the enantiomers are present in equal amounts within each pair.

A new study of the behavioural effects of cyhalothrin in rats was considered. The study did not use entirely standard methods to analyse neurobehavioural effects, but did cover a wide range of relevant neurological end-points. The study used adult male rats to investigate the effects of seven daily oral doses of cyhalothrin at 0 (negative control), 1.0, 3.0 or 7.0 mg/kg bw on clinical signs of toxicity, performance in open-field and plus-maze tests, social interaction and serum corticosterone concentrations. Animals serving as positive controls were treated with picrotoxin, an anxiogenic substance that is a non-competitive GABA_A recep-

tor antagonist. Cyhalothrin and picrotoxin caused similar effects. Signs of intoxication included salivation, tremors and liquid faeces. Behavioural tests showed reduced total locomotor activity in the open field, reduced proportion of time spent in open field central zones, increased immobility time in the open field, reduced proportion of time spent exploring plus-maze open arms, and reduced time spent in social interaction in animals treated with cyhalothrin at ≥ 3 mg/kg bw per day or more. Serum corticosterone concentration was also increased at 3 mg/kg bw per day. The NOEL was 1.0 mg/kg bw per day (Righi & Palermo-Neto, 2003).

Note that the NOEL from the new behavioural study is the same value as the LOEL for the 26-week study in dogs, previously used by the Committee to set a temporary ADI for cyhalothrin.

Comparison of the results of toxicological studies in rats and in dogs gave no reason to suspect that dogs are appreciably more sensitive to cyhalothrin than are rats. It was therefore possible to set an ADI by applying a safety factor to the NOEL of 1 mg/kg bw per day for neurobehavioural toxicity in rats. This dose was also identified as a LOEL for the production of liquid faeces in dogs. As a NOEL had not been identified for dogs, the standard safety factor of 100 was multiplied by an additional safety factor, in order to compensate for this deficiency in the database. A small additional safety factor of only 2 was used as the effect seen in dogs at the LOEL (liquid faeces) was a minor health effect that occurred also in control dogs, as the dose–response relationship suggested that no effect would be evident in dogs at a slightly lower dose, and as there was a clear NOEL for effects in rats. An overall safety factor of 200 was used in the calculation of the ADI.

Therefore:

LOEL in dogs = 1.0 mg/kg bw per day

NOEL in rats = 1.0 mg/kg bw per day

Safety factor = $100 \times 2 = 200$

ADI = 0.005 mg/kg per bw

3. COMMENTS

The present Committee considered the results of a new study of neurobehavioural effects with cyhalothrin and of two new reports of tests for genotoxicity with λ -cyhalothrin, which is the most active of the isomer pairs in cyhalothrin.

The Committee at its fifty-fourth meeting had considered data on the genotoxicity of cyhalothrin and λ -cyhalothrin and concluded that cyhalothrin did not appear to be genotoxic. A range of studies of genotoxicity (tests for reverse mutation in bacteria, cell transformation in vitro, cytogenetic effects in the bone marrow of rats treated in vivo, and for dominant lethal mutation in mice) had given uniformly negative results. A more extensive range of tests for genotoxicity had been performed with λ -cyhalothrin, most of them giving negative results (tests for reverse mutation in bacteria, gene mutation in mammalian cells in vitro, unscheduled DNA synthesis in vitro, cytogenetic effects in vitro, and for micronucleus formation in mice in

vivo). A test for micronucleus formation in fish had given a positive result, but this was disregarded, as the relevance to human health of a positive result in this assay was not known.

One of the new studies of genotoxicity considered by the present Committee was a test for micronucleus formation in fish. Although λ -cyhalothrin gave positive results in this test, it was noted by the Committee that this assay was not validated for use in human risk assessment and again the positive result was disregarded, as the relevance to human health was not known.

A new report described an assay for cytogenetic effects in the bone marrow of rats treated *in vivo*. Increased incidences of chromosomal aberrations in bone marrow cells and of micronuclei in polychromatic erythrocytes indicated that λ -cyhalothrin was genotoxic under the conditions of the assay. It was noted by the Committee that the protocols of these assays in the bone marrow of rats deviated from internationally agreed methodological guidelines, in that small group sizes, extended periods of dosing and late harvest times were used. These deviations could make the tests oversensitive and unreliable. The positive result reported for λ -cyhalothrin in the new assay for cytogenetic effects in the bone marrow of rats *in vivo* was considered in the context of the tests for genotoxicity that had been evaluated at earlier meetings. Considering the negative results of earlier, well-conducted tests with cyhalothrin and λ -cyhalothrin *in vivo* to be more reliable than the positive results of the new study, the Committee concluded that the data as a whole suggested that cyhalothrin presents no genotoxic hazard to humans.

The results of a new series of experiments in rats on the neurobehavioural effects of cyhalothrin administered orally for 7 days indicated a NOEL of 1.0 mg/kg bw per day, with various behavioural changes and increased serum corticosterone concentrations being observed at a dose of 3 mg/kg bw per day. The Committee noted that the NOEL for this study was the lowest NOEL for toxicological effects in rats and was numerically the same value as the LOEL for liquid faeces in dogs, which had been used to set the temporary ADI for cyhalothrin.

4. EVALUATION

Comparison of the results of the studies of toxicity in rats with those in dogs suggested that cyhalothrin is of similar toxicity in the two species. The Committee decided that the temporary ADI could be replaced by an ADI of 0–0.005 mg/kg bw, which was determined by dividing the LOEL of 1 mg/kg bw per day for dogs (also the NOEL for rats) by a safety factor of 200. The safety factor incorporated a factor of 2 to compensate for the absence of a NOEL for dogs. An additional factor was considered appropriate because: liquid faeces is a common minor health effect in dogs, and some liquid faeces also occurred in control dogs; the LOEL was close to a NOEL; and because there was a clear NOEL for neurobehavioural effects in rats.

5. REFERENCES

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