Comment (2) Company-Manufacturer on the proposed classification of Metaldehyde

CLH report, Proposal for Harmonised Classification and Labelling
Based on Regulation (EC) No 1272/2008 (CLP Regulation)
Annex VI, Part 2.
Version No. 06; 26.02.2016

Metaldehyde: review of findings for STOT RE

Background

The CLH report for metaldehyde (version 06, dated 26.02.2016) concludes on classification for systemic target organ toxicity following repeated exposure (STOT RE, H373). The basis for this conclusion is "mortality occurring at 30 mg/kg bw/d in a 52-week dog study and histopathological testes findings observed at 60 and 90 mg/kg bw/d in a 26-week and 52-week dog study". The purpose of this review is to evaluate the treatment-relatedness of these findings as a trigger for classification.

Conclusions

The salient aspects of the two studies are given below. Our weight-of-evidence view is that the effects seen at high dose levels cannot be considered as scientifically sound and thus casts sufficient doubt upon the overall conclusion of classification being required.

In the 52-week study there were two (of eight) mortalities in the mid-dose group and one (of eight) in the high-dose group. The pathology report concluded on treatment-relatedness of the findings and speculated that the cause of death was secondary to pulmonary exposure as a result of emesis. The lack of strong dose-response also supports the conclusion that mortality is not a primary response to treatment with metaldehyde, but more likely a secondary response to some other response that itself may or may not be related to treatment. Without definitive evidence for a causal relationship it is not appropriate to conclude that mortality is directly related to treatment with metaldehyde.

In both the 26- and 52-week studies, there was evidence for a slight shift in severity, but not incidence, of germinal epithelium atrophy and degeneration at doses of 60 and 90 mg/kg/day. Although the high-dose was not associated with signs of adverse response in the earlier 26-week study, the data presented for the 52-week study and its pilot study call into question whether this dose level should have been used in the first place, other than that it had been used in the prior 26-week study. That two studies conducted at the same facility, in the same species and strain of

animal, albeit more than twenty years apart, can result in such different tolerance reactions is puzzling. But the earlier study was not conducted to GLP, and evidence from the later study strongly indicates that 90 mg/kg/day, and even 60 mg/kg/day, metaldehyde exceed current expectations for a maximum tolerated dose. As such, the effects noted at these dose levels are not safe and should be discounted from the evaluation.

26-week repeated dose toxicity in beagle dogs

These studies predated the introduction of GLP and OECD test guidelines. The main study, however, was conducted to the proposed US EPA guideline for the testing of pesticides (dated 22 August 1978). The test article was administered in the diet, which was prepared on a daily basis, but which was not analysed to verify test article concentration or homogeneity. The diet was, however, administered in such a way as to ensure complete ingestion of the test article (Neumann, 1991).

A preliminary 14-day pilot study was conducted to establish doses for the main study (Neumann, 1991). In this pilot study, one male and one female dog were administered 90 or 180 mg/kg/day metaldehyde (minimum 99% purity) in the diet. No signs of intolerance were noted at the lower dose level. Both dogs given the higher dose level showed moderate tremor and ataxia lasting 1–2 hours on the first day of treatment, the male showed salivation while the female vomited within 1 hour. On the second day, the male showed repeated tonic convulsions within one hour of feeding, showed increasing sedation over 6 hours, and died in a comatose state. The female vomited repeatedly for more than 3 hours from 1½ hours after feeding. Dosing of 180 mg/kg/day metaldehyde was discontinued.

In the main study, groups of six male and six female dogs were administered metaldehyde in the diet at dose levels of 0, 20, 60, or 90 mg/kg/day (Neumann, 1980, 1991). Animals were eight months of age at commencement. Over the period of administration, there were no clinical or behavioural signs or changes to food and water consumption or body weight gain that were attributed to treatment. At necropsy, there were no unusual macroscopic findings, or effects upon organ weights. Histopathology evaluation suggested slight degenerative changes in the liver and prostate, primarily hydrophobic enlargement of Kupffer cells and prostate atrophy, which were reported to be “within the normal limits” (Neumann, 1980). Subsequent re-examination of the testes was conducted to include severity of findings, and identified the following as treatment-related effects: cryptorchism and moderate testicular atrophy and a mild to moderate focal atrophy of the germinal epithelium in the mid-dose group; mild to moderate diffuse atrophy of the germinal epithelium in the high-dose group (Leuschner, 2009).

The identification of cryptorchidism, and the associated testicular atrophy, as a treatment-related effect in the mid-dose group is untenable for three reasons: the same effect, at the same incidence, was noted in the control group; the same effect was not noted in the high-dose group; and testis descent in beagle dogs is generally completed by two months of age, that is six months before the animals were placed on study. Therefore, these findings are incidental and unrelated to treatment. Atrophy of the germinal epithelium was observed at similar incidence in all groups, although there was a general shift from focal to diffuse atrophy at the high-dose and in severity from minimal to mild/moderate at the mid- and high-dose levels (Table 1).

52-week repeated dose toxicity in beagle dogs

A preliminary study was conducted in two phases to establish doses for the main study (Leuschner, 2002). In the first phase, two male and two female dogs were administered 90 mg/kg/day metaldehyde (98.3% purity) in the diet. Following the administration of the first dose there were severe adverse reactions in some or all, including ataxia, clonic convulsions, emesis, lateral position, difficulty in breathing, head shaking, and one animal had to be euthanised moribund. After the three-day wash-out period, the animals were administered escalating dose levels of 30, 60, 75, and then 90 mg/kg/day metaldehyde, each for a period of three days with a two-day wash-out period before each escalation. Severe clinical signs were noted at doses of 60 mg/kg/day and above, including ataxia, clonic convulsions, emesis, tremors, mydriasis, increased respiratory rate, and reduced motility. In the second phase, groups of two males and two females were administered 75 or 90 mg/kg/day metaldehyde for a period of four weeks. Adverse effects at the lower dose level included slight tremor, emesis, inflated stomach, and increased respiratory rate, lasting for up to six hours from 1–2 hours after administration, although these signs reduced over time and were not noted from day 7 onwards. Similar effects, along with slight ataxia, and lateral and abdominal position, were observed at the high dose, although the period of onset after dosing was shorter and the reversal of findings was prolonged such that “signs of toxicity had almost disappeared towards the end of the 4-week treatment”.

In the main study, groups of four male and four female dogs were administered metaldehyde in the diet at dose levels of 0, 10, 30, or 90 mg/kg/day (Leuschner, 2004). No behavioural changes were noted at the low- and mid-dose levels, but at the high-dose level one or more animals exhibited ataxia, reduced motility, emesis, tremor, twitching, and salivation; the incidence and severity of which reduced from the nineteenth week of treatment. Body weight gain showed an apparent treatment-related reduction, by up to 50% in high-dose males compared to control, although the differences were not statistically significant. One male and one female of the mid-dose group, and

one female of the high-dose group were found dead during weeks 46, 40, and 37 respectively, without pre-mortal symptoms. Post-mortem examination suggested that none of the macroscopic or microscopic changes in the mid-dose male were associated with death, and that moderate interstitial or bronchopneumonia in the females may have contributed to the deaths of the females but were not caused by metaldehyde. The pathology report did, however, conclude that the deaths were treatment-related and speculated that the cause of death may be secondary to intratracheal effusion of test article during emesis.

Mild to moderate focal atrophy or degeneration of the germinal epithelium was observed in the high-dose group (Table 1).

References

Leuschner J. (2002). 4-Week Dose-Range-Finding Study for a 52-Week Chronic Toxicity Study of Metaldehyde by Oral Administration via the Diet to beagle Dogs – According to OECD Guideline 452. LPT Laboratory for Pharmacology and Toxicology KG, Hamburg, Germany. Lonza report number 3505.

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Leuschner J. (2009). Histological Re-examination of the Testes and Re-evaluation of the Findings of the 26-Week Toxicity of Metaldehyde 99% in Beagle Dogs after Oral Administration (LPT Study report dated March 31, 1980). LPT Laboratory for Pharmacology and Toxicology KG, Hamburg, Germany. Lonza report number 4376.

Neumann B.-W. (1980). 26-Weeks-Toxicity Study of Metaldehyde 99% - Called “Metaldehyd” - in Beagle-Dogs After Oral Administration. LPT Laboratory for Pharmacology and Toxicology KG, Hamburg, Germany. Lonza report number 1379 Part 1.

Neumann B.-W. (1991). 26-Week Toxicity Study of Metaldehyde 99% - Called ‘Metaldehyde’ - in Beagle Dogs After Oral Administration. Supplement No 1. LPT Laboratory for Pharmacology and Toxicology KG, Hamburg, Germany. Lonza report number 1379 Part 2.

Table 1: Incidence and severity of germinal epithelium lesions in beagle dogs treated with metaldehyde in the diet for 26 or 52 weeks. Groups I–IV, control, low-, mid-, and high-dose groups respectively; #, incidence; Sev., average severity grade; *, includes one animal premature dead. Severity grades for the 26-week study are also presented following weighting for pattern of findings; effects described as 'focal' were weighted by subtraction of 0.75 from the severity score, while effects described as 'diffuse' were unmodified as the modifier scheme does not include 'diffuse' as a modifier descriptor (Leuschner, 2009).

		Group I		Group II		Group III		Group IV	
		#	Sev.	#	Sev.	#	Sev.	#	Sev.
26-week									
Number evaluated		6		6		6		6	
Testis I:	atrophy (focal)	4	0.67	2	0.50	3	1.33	1	0.17
	atrophy (diffuse)	0		0		0		4	1.67
Testis II:	atrophy (focal)	2	0.33	2	0.50	4	1.50	1	0.17
	atrophy (diffuse)	0		0		0		4	1.83
26-week (weighted)									
Testis I:	atrophy	4	0.17	2	0.25	3	1.13	5	1.71
Testis II:	atrophy	2	0.08	2	0.25	4	1.08	5	1.88
52-week									
Number evaluated		4		4		4*		4	
Testis I:	atrophy	0		0		2*	0.88	2	1.31
	degeneration	0		1	0.31	0		3	1.81
Testis II:	atrophy	0		0		2*	0.13	3	2.06
	degeneration	0		1	0.31	0		3	1.81

Basel, 22nd September 2016