A.3.10. Reproductive toxicity

A.3.10.1. Sexual function and fertility

No comment.

A.3.10.2. Developmental toxicity

Section A.3.10.2. gives a thorough overview of relevant studies on the developmental toxicity endpoint. We agree with the conclusion that classification of sodium pyrithione for this endpoint is not supported by the available data. There are, however, some points to address for the two PNDT studies in rats.

For the oral PNDT study, table A.82 and A.83(a)(iii) state that the incidences of incomplete ossification of 6th sternebrae and metatarsals was increased in the high dose group (positive for dose-response). The study report did not present statistical analyses of these endpoints, but simply noted (section 5.11) that "The only sign of toxicological relevance was the increased incidence of incomplete ossification of 6th sternebrae…and metatarsal(s) observed in high-dose foetuses compared to controls". However, the incidences of these two endpoints were not found to be statistically significantly different from the control group (p>0.05) in a subsequent analysis (Appendix 1). The only foetal endpoints that were statistically significantly changed in the high-dose group were litter and foetal weight, incidence of small foetuses, and incidence of foetuses with absent ossification of the metacarpal(s).

Analysis of covariance has been applied to the foetal weight data, with adjusted maternal body weight gain (weight gain throughout gestation adjusted for the weight of the gravid uterus) and live foetuses per litter as dependent covariates. This analysis showed no significant treatment-related effects compared to control, while the influence of adjusted maternal body weight gain in the model was significant (p<0.05). Similarly, nested logistic regression of small foetuses and missing ossification of metacarpals with adjusted maternal weight gain as dependent covariate showed no significant treatment-related effects compared to control, but a significant influence of adjusted body weight gain (p<0.05).

These analyses further support the conclusion that reduced foetal weight, small foetuses, and absent ossification were secondary to maternal toxicity.

With regards to the dermal PNDT study, and as noted in the CLH proposal, all effects in the offspring were only observed in the high-dose group (7 mg/kg bw/day). This dose level also induced maternal toxicity (5/25 maternal mortalities) that clearly exceeds the general 10% mortality limit recognised in the current OECD TG 414 and CLP Regulation (3.7.2.4.4):

> “Maternal mortality greater than 10 % is considered excessive and the data for that dose level shall not normally be considered for further evaluation.”

Indeed, the study report acknowledges, “This dosage level is considered excessively high for a teratology study.”

If the high-dose group is excluded from the assessment, there were no identified effects upon the progeny. The assessment of this study would be strengthened by reference to the accepted limit of mortality as given in the CLP Regulation.

A.3.10.3. Effects on or via lactation

No comment.

Appendix 1

Statistical analysis of foetal effects reported in a prenatal developmental toxicity study of sodium pyrithione in rat

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Background

A prenatal developmental toxicity study of sodium pyrithione administered by oral gavage to time-pregnant rats reported adverse findings at the high-dose level of 4 mg/kg/day. These findings have been summarised by the dossier submitter in Tables A.82, A.83(a)(ii), and A.83(a)(iii) as reduced foetal weight, reduced litter weight, increased incidence of small foetuses, and increased incidences of foetuses with skeletal anomalies, specifically incomplete ossification of 6th sternebrae, metacarpals, and metatarsals. This dose level was also associated with reduced food consumption and body weight gain in the dams.

While statistical analysis of litter and foetal weight was presented in the study report, such analyses for external and skeletal anomalies was not presented.

The purpose of the analysis presented herein is twofold: to investigate the statistical significance of external and skeletal anomalies; and to investigate the possible role of reduced maternal body weight gain as an explanatory factor in impaired foetal growth and development.

Methods

Data were abstracted from the study report (Cicalese, 2002). Data were analysed by the following methods using Stata 14.2 (StataCorp LLC, College Station, TX).

Foetal and litter weights were analysed with Bartlett's test for homoscedasticity, analysis of variance (ANOVA), and Dunnett's test; the role of adjusted maternal body weight gain (ABWG; weight gain over the course of gestation minus the weight of the gravid uterus) and, in the case of foetal weight, litter size was investigated as dependent covariates in analysis of covariance (ANCOVA).

External and skeletal anomalies were analysed by nested logistic regression. When regression analysis identified a significant treatment component, it was repeated with adjusted maternal body weight gain as explanatory dependent covariable. In all cases, the level of significance was established as α=0.05.

Results

Litter and foetal weight

Statistical analyses of litter and foetal weight are summarised in Table 1. Both litter and foetal weights were reduced in the high-dose group compared to the control group. Neither were found to be statistically significantly different from control when the influence of

ABWG was included in the model. The influence of ABWG in the model was statistically significant (p=0.002, litter weight; p=0.000, foetal weight).

Table 1. Calculated p values compared to control following analysis of litter and foetal weight by ANOVA and ANCOVA with ABWG (a) and live litter size (b) as covariates.

Group	Litter weight		Foetal weight	
	ANOVA	ANCOVA (a)	ANOVA	ANCOVA (a,b)
2 (1 mg/kg/day)	0.996	0.985	0.760	0.619
3 (2 mg/kg/day)	0.803	0.998	0.940	0.979
4 (4 mg/kg/day)	0.001*	0.983	0.000*	0.994

* p<0.05

Small foetuses

Statistical analysis of small foetuses summarised in Table 2. The incidence of small foetuses was increased in the high-dose group compared to the control group, but was not found to be statistically significantly different from control when the influence of ABWG was included in the model. The influence of ABWG in the model was significant (p=0.000).

Table 2. Calculated p values compared to control following analysis of the incidence of small foetuses by nested logistic regression (a) excluding and (b) including ABWG as covariate.

Group	Regression (a)	Regression (b)
2 (1 mg/kg/day)	0.276	0.237
3 (2 mg/kg/day)	0.118	0.329
4 (4 mg/kg/day)	0.007*	0.467

* p<0.05

Skeletal anomalies

Statistical analyses of foetuses with absent ossification of the 6th sternebrae, metacarpals, or metatarsals are summarised in Table 3.

The incidences of foetuses with absent ossification of the 6th sternebrae or metatarsals were not statistically different between treatment groups and the control group.

The incidence of foetuses with absent ossification of the metacarpals was significantly higher in the high-dose group compared to the control group, but was not statistically significantly different from control when the influence of ABWG was included in the model. The influence of ABWG in the model was significant (p=0.039).

Table 3. Calculated *p* values compared to control following analysis of the incidence of absent ossification by nested logistic regression (a) excluding and (b) including ABWG as covariate.

	6th Sternebrae	Metacarpals		Metatarsals
Group	Regression (a)	Regression (a)	Regression (b)	Regression (a)
2 (1 mg/kg/day)	0.514	0.471	0.405	0.812
3 (2 mg/kg/day)	0.586	0.548	0.719	0.500
4 (4 mg/kg/day)	0.160	0.001*	0.654	0.320

* $p<0.05$

Conclusion

Litter weight, foetal weight, and four external or skeletal anomalies have been reported as having a dose relationship to treatment with sodium pyrithione. Only two of these findings, litter and foetal weight, were reported with statistical analysis in the original study report (Cicalese, 2002). The analysis has been extended to include the four anomalies, and to investigate the role of ABWG as a potential explanatory factor in these findings.

Two skeletal anomalies, absent ossification of the 6th sternebrae and metatarsals, were not found to be significantly influenced by treatment at any dose level ($p>0.05$). The decrease in litter and foetal weights, and increased incidences of small foetuses and foetuses with absent ossification of the metacarpals, was confirmed. Inclusion of ABWG in the model showed no treatment-related differences, and ABWG was a significant explanatory variable.

The findings confirm the original conclusion that effects noted in the progeny can be explained as a secondary response to reduced weight gain in the dams.