



Discussion of the hydrocephaly incidence in the rabbit developmental toxicity study conducted on flutianil

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Sporadic increases in hydrocephaly within New Zealand White rabbits kept under laboratory conditions is not uncommon.

The incidence of hydrocephaly observed in rabbit developmental study presented in the CLH dossier was deemed to be sporadic in nature, and not test-article related. Such incidences of sporadic increases in hydrocephalus have been reported in the public domain. Hanley *et al* [1] reported an increase in the spontaneous incidence of central nervous system (CNS) malformations in the control rabbit population in rabbit teratology studies. In five studies conducted, a total of 11 control fetuses with these same CNS anomalies were reported (5 having severely dilated lateral ventricles and 6 with hydrocephalus), 4 of which occurred in a single study with a control size of 132 (3%) and another 3 in a separate study with a control group of 138 fetuses. The historical control incidence ranged from 0 – 2.2% of control fetuses with CNS malformations over a 2 year period.

In a compendium of historical control data compiled by the Middle Atlantic Reproduction and Teratology Association (MARTA) and the Midwest Teratology Association (MTA) from developmental studies conducted between 1992 and 1994 using the New Zealand White rabbit, the average fetal incidence of hydrocephalus was 0.14%. However, individual studies with control incidences as high as 5.2% were reported, while litter incidences of 1.7% (average) and 50% (upper limit) were seen ([2] cited in [1]). Dilated cerebral ventricles were reported separately in this compendium, with an average fetal incidence of 0.4%, and incidences as high as 14.1% in individual studies. Kalter [3] described epidemic-like waves of hydrocephalus in various strains of rabbit; one wave initially reported in 1940 lasted approximately 2 years, with an overall incidence of hydrocephalus of 143 in 810 fetuses examined, while a second wave reported in 1966 had a 14.4% incidence (148/1103) of hydrocephalus, without any explanation on a hereditary, infectious or nutritional basis. It is therefore evident that CNS anomalies have been reported in the public domain with incidences within control populations similar to those seen in the high-dose group in this present study.

Although a marginal increase in fetal incidence was observed, when interpreting such data, the appropriate unit for evaluation is litter incidence. Litter incidence did not differ from the laboratory's historical control range. The hydrocephalus observed within this study occurred in the absence of any other treatment related CNS anomalies suggesting neural tube development as a unique target, such as exencephalus or spina bifida was not a unique target. Also there were no other treatment-related increases in malformations in any other organ system and no evidence of any delayed maturation such as fetal body weight reductions or skeletal ossification to suggest any adverse effect. It is also important to mention that with known teratogens, such as *N*-methyl-*N'*-nitro-*N*-nitroso-guanidine (MNNG), malformations are numerous and not limited [4], as seen in this study.

ADME data reported in the CLH dossier confirms that oral absorption appeared to be saturable. At low doses (10 mg/kg/bw) oral absorption was estimated to be 18%, (decreasing to 5% and 2% at higher doses (250 and 1000 mg/kg bw, respectively). Therefore, if the doses within the rabbit study are corrected for oral absorption, systemic dose levels of 0, 5, 15 and 20 mg/kg bw/day are obtained. For an effect to be test article related, an increase in hydrocephaly at the intermediate dose (1.3-fold lower than the high dose, when corrected to account for oral absorption) would have been expected. No incidence in hydrocephaly was observed.

It can therefore be concluded that in the absence of any additional effects in rabbit fetuses in this study along with a lack of other CNS malformations following exposure to flutianil, the increased incidence of hydrocephalus observed in 3 fetuses limited to 1 litter is consistent with the laboratory's historical control litter incidence range, with further support from the public



domain data. The increased incidence in visceral hydrocephalus is therefore deemed to be sporadic in nature, with its relationship to test article administration questionable.

There were no statistically significant differences in the number of variations in the treated groups compared to the control.

In maternal females no significant treatment related findings were observed, the maternal NOAEL was therefore deemed to be 1000 mg/kg bw/day.

In offspring no significant treatment related findings were observed, the developmental NOAEL was therefore deemed to be 1000 mg/kg bw/day.

Summary and discussion of reproductive toxicity

In the main rabbit developmental study the total number of malformations observed in the treated groups were comparable to that of the concurrent control. In the high dose group three fetuses in the same litter had visceral hydrocephaly. Whilst the fetal incidence exceeded the laboratory's historical control range, the litter incidence was consistent with the laboratory range. Litter incidence is the more appropriate unit for evaluation in developmental studies. Furthermore, when administered dose levels were corrected for oral absorption the lack of dose response was more evident.

Public domain data further supports this observation that sporadic increases in hydrocephaly within New Zealand White rabbits kept under laboratory conditions are not uncommon, with incidences up to 3% in studies conducted at a similar time to this study. Older data report epidemic-like waves of hydrocephalus in various strains of rabbit, without any explanation.

The hydrocephalus observed within this study occurred in the absence of any other treatment related CNS anomalies such as exencephalus or spina bifida, which would suggest neural tube development as a unique target. Also there were no other treatment-related increases in malformations in any other organ system and no evidence of any delayed maturation such as fetal body weight reductions or skeletal ossification to suggest any adverse effect. It is also important to mention that with known teratogens, such as thalidomide, malformations are numerous and not limited, as seen in this study.

It can therefore be concluded that in the absence of any additional effects in rabbit fetuses in this study along with a lack of other CNS malformations following exposure to flutianil, the increased fetal incidence of hydrocephalus observed (3 fetuses limited to 1 litter) marginally exceeded the laboratory's historical control range, but when assessed against the litter incidence (the more appropriate unit for evaluation), is consistent with the laboratory's historical control range and public domain data. The increased incidence in visceral hydrocephalus is therefore deemed to be sporadic in nature, with its relationship to test article administration questionable.

There were no statistically significant differences in the number of variations in the treated groups compared to the control.

Comparison with criteria

There was no convincing evidence that flutianil has any fertility or developmental effects in rats or rabbits. The criteria for classification for reproductive toxicity were not met.



Reference

<i>(a Data Protection Claimed (Y/N) according to Regulation (EC) 1107/2009)</i>		a
1	Hanley Jr., T.R., Carney, E.E. & Johnson, E.M. (2000). Developmental toxicity studies in rats and rabbits with 3,5,6-trichloro-2-pyridinol, the major metabolite of chlorpyrifos. <i>Toxicol. Sci.</i> , 53 , pp 100-108	N
2	MARTA/MTA (1996). <i>Historical control data (1992-1994) for developmental and reproductive toxicity studies using the New Zealand White rabbit</i> . Compiled by MARTA (Middle Atlantic Reproduction and Teratology Association) and MTA (Midwest Teratology Association), HRP. Inc. Cited in [1]	N
3	Kalter, H. (1968). Spontaneous malformations: rabbit. In <i>Teratology of the central nervous system.</i> , pp 244-255. University of Chicago Press, Chicago. Cited in [1]	N
4	Shepard, T.H. (1998). <i>Catalog of teratogenic agents</i> , 9th Edition, The Johns Hopkins University Press, Baltimore. Cited in [1]	N