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MEMORANDUM

October 13, 1999

SUBJECT: D259256 CARBARYL (056801) Developmental Neurotoxicity Study
Rhône-Poulenc Response to EPA Comments about Morphometric Measurements

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The purpose of this memorandum is to review the response of Rhône-Poulenc to a request made by HED in its review of a Developmental Neurotoxicity Study (MRID 44393701) of Carbaryl. HED asked the registrant to conduct morphometric measurements of animals in the mid dose and possibly low dose groups, based on the observation that statistically significant effects on a number of morphometric measures were seen in high dose animals and, because, without evaluation of the lower dose groups, an NOAEL for these effects would be difficult to establish.

CONCLUSION

The registrant was unable to examine the mid dose animals as requested by EPA due to inadequate number of control animals and differences in tissue preservation between treated and control rats. Thus, this remains a study deficiency.

The re-assessment uncovered errors in some measures and confirmed others. The additional statistical analyses which attempted to account for multiple comparisons, rendered the apparent number of statistically significant findings far fewer, but some findings including data on pup cerebellar length remained statistically significant.

The decrease in female pups given 10 mg/kg in the length of the cerebellum is still regarded as a treatment related effect.

INTRODUCTION

In our limited experience with these studies (roughly 12), HED would first acknowledge that the study laboratory exerted greater diligence than other study laboratories in their conduct and reporting of these findings. These measures are viewed by EPA as quite important, because they represent an attempt, however simple, to address the growth or development of these structures, rather than the histological examination of tissues that is essentially equivalent to that done for adult animals.

The registrant reports that it was unable to conduct morphometric analyses of the mid dose animals. There were insufficient control animals for a new evaluation; existing control slides were prepared after a brief storage in fixative, while mid and low dose animals had been stored in fixative for 2 years; since the degree of shrinkage from such long term fixative storage is indeterminate, comparisons between controls and mid and low dose animals could not be made. The failure to examine mid dose animals after statistically significant findings were seen in a variety of measures in high dose animals was listed as a study deficiency in our review. The unfortunate failure to preserve the tissue in such a way that such measures could be carried out later means it must remain a deficiency.

Instead, a re-examination of the original high dose slides was made and additional statistical analyses conducted. The review of the findings from these activities will be the subject of the remainder of this memorandum.

RESULTS

Some type of peer review procedure, which would required that the reader be unaware of the treatment group of the examined slide, as is now required for re-reads of slides in carcinogenicity studies is the preferred method for re-evaluation of data (See e.g., OPP PR notice 94-5 for re-evaluation of carcinogenicity studies). Re-evaluation without awareness of dose group is also described in the subjective analysis section of the developmental neurotoxicity guideline with reference to neuropathological data. This re-analysis was not performed in this way.

ORIGINAL STUDY REPORT

The principal findings in the morphometric measurements in the original study report were the findings in the cerebellar measures in the high dose females. In pups assessed on day 11, there were statistically significant decreases of 15-22% in right and left cerebellar length (Line F), respectively. While smaller and not statistically significantly different from control animals, cerebellar weights in the high and mid dose female pups were somewhat, 5-10%, smaller, respectively, in comparison to controls as measured on day 11. Cerebellar weights were not measured on day 70.

In adult females from the high dose group, the width of the cerebellum (Line G) was increased 19-30% for right and left cerebellum, respectively. While a different measure, the continued distortion of the shape of the cerebellum in this group added to the concern for these effects.

REASSESSMENT

In the repeat evaluation of these tissues, in pups, a 14-18% decrease in Line F was found for right and left cerebellum, basically confirming the original measurements.

The submitter asserts that these findings are of limited significance because they were only found in one sex. But a lack of concordance between the sexes in general is not considered a cogent argument to discount a finding, and there is no specific reason cited here to dispute that view for this effect. Thus, this difference in females in the length of the cerebellum is still regarded as a treatment related effect

In the repeat evaluation of these tissues in rats on day 70, 8-15% INCREASES in Line G were found, the opposite of what was seen in the earlier evaluations. It was further asserted that the original measures that showed such a large decrease were based on erroneous measurements and should not be considered further. The other evaluations of these tissues in other animals also support the lack of effect.

The submission also contains additional statistical analyses which applied a PROC Multtest (SAS Release 6.12) procedure as a means to correct for multiple comparisons, that is, the large numbers of measures made and the chance for false positives. This approach uses a large number of simulations based on the study data and results in the adjustment of the p values that were previously reported by Dunnet's test. The results of these analyses are that the adjusted p values are generally an order of magnitude higher than previously reported.

Without rejecting these findings explicitly, 2 observations should be noted. First, there are 6 animals/dose and 2 dose groups for these measures, a very small number in the realm of statistical analyses. Second, this analysis is provided in a situation in which a minimized finding suits the source. It is *post hoc,* and one might say, *propter hoc* as well. Would the submitter find such an analysis as compelling if made of the sufficiency of a body weight gain depression that defines the adequacy of the high dose in this or other studies? Such analyses are rarely, if ever conducted in those situations. That is why such analyses are best conducted prior to the study and not after.

Despite this, the measures of the left cerebellum in female pups was still statistically significant from both the original and repeated evaluations for the left cerebellum (from 0.002 to 0.02 and 0.007 to 0.04), while the p values for the right cerebellar measures were deemed to be higher than 0.05 and thus no longer statistically significant (from 0.04 to 0.16; and from 0.02 to 0.2)(unadjusted to adjusted p values, respectively).

In HED's judgment, the weight of the evidence would support continued reliance on the changes in the female high dose pups in cerebellar length as treatment related, by virtue of their

repeated measurement by the study technician as an effect, by the magnitude of the effect, and by the partial replication of the statistical signficance of these findings, even with a much more conservative statistical analysis.

The most productive way to proceed in the future in general is:

1. the timely evaluation of all dose groups when treatment related effects **arguably appear** treatment related;
2. to set out the most powerful statistical analyses *a priori*;
3. to resolve this type of issue by peer review procedure, when necessary; and
4. with more data on more animals otherwise.