CLH – Proposal for Harmonised Classification and Labelling of Metam for Reproductive Toxicity

Independent Opinion by [redacted], Toxicologist

Introduction

Metam is currently undergoing both an EU renewal of approval under Regulation (EC) 1107/2009 (PPP Regulation) as well as the harmonised classification procedure under the Regulation (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures (CLP Regulation) to determine, among others, whether Metam should be classified as carcinogenic, mutagenic, or reprotoxic (CMR). The RMS (Belgium) has proposed that Metam should be classified as Repr. 2 (H361d). This document is an independent review of the reproductive toxicity data on Metam to determine whether there is a justification, or not, that supports the opinion of the RMS.

Reproductive toxicity is a term that includes several elements; effects on sexual function and fertility, together grouped as effects on reproduction; and effects of developmental toxicity. It is accepted by all stakeholders that Metam has no adverse effects on sexual function, fertility, or on reproduction in general, so these elements will not be discussed further. However, there is disagreement on whether Metam has the potential to cause developmental toxicity, and this is the focus of this document.

CLP Guidance on Reproductive and Developmental Toxicity

Prior to a review of the data, it is informative to consider the European guidance information. The CLP guidance document (Version 5.0-July 2017) defines developmental toxicity as, "*Developmental toxicity includes, in its widest sense, any effect which interferes with normal development of the conceptus, either before or after birth, and resulting from exposure of either parent prior to conception, or exposure of the developing offspring during prenatal development, or postnatally, to the time of sexual maturation. However, it is considered that classification under the heading of developmental toxicity is primarily intended to provide a hazard warning for pregnant women, and for men and women of reproductive capacity. Therefore, for pragmatic purposes of classification, developmental toxicity essentially means adverse effects induced during pregnancy, or as a result of parental exposure. These effects can be manifested at any point in the life span of the organism. The major manifestations of developmental toxicity include (1) death of the developing organism, (2) structural abnormality, (3) altered growth, and (4) functional deficiency.*"

The classification of a substance in the presence of parental toxicity is discussed in section 3.7.2.2.1 of the CLP guidance document, and states "*In general all findings on reproductive toxicity should be considered for classification purposes irrespective of the level of parental*

toxicity. A comparison between the severity of the effects on fertility/development and the severity of other toxicological findings must be performed."

It is implicit that the severity of developmental effects observed in the foetuses of developmental toxicity studies should be compared with other toxicological findings, in particular the effects observed in the parents. A weight of evidence approach should be used to conclude whether effects in the offspring are secondary to non-specific mechanisms related to stress and the disruption of maternal homeostasis. The guidance on CLP provides some key statements relating to the weight of evidence approach:

"*Developmental effects which occur even in the presence of maternal toxicity are considered to be evidence of developmental toxicity, unless it can be unequivocally demonstrated on a case-by-case basis that the developmental effects are secondary to maternal toxicity*."

And

"*Classification is not necessarily the outcome in the case of minor developmental changes, when there is only a small reduction in foetal/pup body weight or retardation of ossification when seen in association with maternal toxicity*."

Therefore, it is clear that the relative severity of the maternal toxicity and the severity of the foetal effects is critical to determine whether classification may be justified or not. Maternal effects that may be considered in such a severity analysis include mortality, body weight and body weight change, food and water consumption, clinical evaluations, observations on clinical signs, and post-mortem observations.

Developmental Toxicity Studies on Metam

There are four main studies on the reproductive and developmental toxicity of metam-sodium. There are two prenatal toxicity studies in the rat (Hellwig and Hildebrand, 1987; Tinston, 1993) and two in the rabbit (Hellwig, 1987; Hodge, 1993).

OECD 414 in the Rat – Hellwig & Hildebrand, 1987

In this study, female rats were exposed to metam-sodium at 0, 10, 40, and 120 mg/kg/day on days 6-15 of gestation. In the DRAR the RMS state that "*No treatment related clinical signs of toxicity were observed. Clear evidence of maternal toxicity was seen at 40 and 120 mg/kg/day dose levels of metam-sodium as shown by dose-related reductions in bodyweight, bodyweight gain, and food consumption during the dosing period. Thus the NOAEL for maternal toxicity was considered to be 10 mg/kg/day."* Evidence for foetotoxicity was observed at 40 and 120 mg/kg/day as reduced foetal and placental weights, as well as retarded ossification of foetal skeletons. According to the authors of the study, the NOAEL for embryotoxicity was considered to be 10 mg/kg/day and the NOAEL for developmental toxicity was also considered to be 10 mg/kg/day.

The analysis of the study by the RMS showed that there was no effect at the low dose level of 10 mg/kg bw/day with respect to generalised retardations of the skeleton (all effects merged). But, when evaluated individually, some variations were observed at a higher frequency in the low dose group than in the control group. The RMS concluded that the low dose may be considered as a LOAEL rather than a NOAEL. This conclusion seems to be rather illogical, because if the total number of variations in foetuses and litters is the same in the control and low dose groups, then, if some individual types of variations are higher in the low dose than in the control, then the reverse must be true for other types of anomalies. Indeed, for example, this pattern is what was observed for vertebral column variation; the incidence of BHKU (L) was 58.3% in control and 47.8% in low dose, whereas BNKU (L) was 33.3 and 52.2% respectively. BHKU and BNKU are near identical variations (BHKU - Thoracic vertebra body dumbbell-shaped, cartilage unchanged; BNKU - Thoracic vertebrae bodies dumbbell-shaped, cartilage unchanged; L – litter). However, the RMS identifies the increase in BNKU as being an effect "*with probable involvement of the treatment with the a.s*." and justifies this conclusion on the basis that the increase is part of a dose-related trend. However, reverting back to the CLP guidance and the severity of the effects as part of a weight of evidence evaluation, it seems clear that the effects identified by the RMS are not severe but are merely minor variations and retardations. Furthermore, it is difficult to envisage a toxicity mode of action that may selectively induce BNKU and at the same time reduce the incidence of BHKU.

OECD 414 in the Rat – Tinston, 1981

In this study, female rats were exposed to metam-sodium at 0, 5, 20, and 60 mg/kg/day on days 7-16 of gestation. Contrary to the study in rats of 1987, where no clinical signs were observed, even up to the highest dose level of 120 mg/kg/day, in this study many clinical signs were observed at all dose levels, although relatively minor at the low dose but increasing in incidence and severity with dose. Clear evidence of maternal toxicity was seen at 5, 20 and 60 mg/kg/day dose levels of metam-sodium as shown by dose-related reductions in bodyweight, bodyweight gain, and food consumption during the dosing period. It is difficult to understand why both the applicant and the RMS conclude that maternal toxicity was evident at only the mid and high dose levels and that the NOAEL for maternal toxicity was 5 mg/kg/bw, based on a dose related reduction in bodyweight gain and food consumption. A conclusion that the RMS chose to maintain at renewal. However, the data show that statistically significant reductions in food consumption were observed during the dosing period in **all three** dose groups. Furthermore, statistically significant reductions in bodyweight were observed in **all three** dose groups and that actual **bodyweight loss** was observed at gestation day 10 in **all three** dose levels. Statistically significant reductions in the body weight of the females were observed in all three dose groups on gestation days 12 and 16 compared to the control group. Clearly this study does not meet the requirements of OECD 414, where it is stated that the "…*lowest dose level should not produce any evidence of either maternal or developmental toxicity*.", because maternal toxicity was evident at all three dose levels.

In terms of skeletal variants, the RMS identifies "*elevated incidences of unossified odontoid, cervical vertebrae and calcaneum (variants) as well as a minor defect in a cervical vertebrae*

center at 5 mg/kg b.w./d onwards" as a justification to reject the previously accepted developmental NOAEL of 5 mg/kg/day and to adopt <5mg/kg/day instead. The RMS argues that a trend towards ossification delays supports the observation of top-dose malformations and justifies a proposal to classify metam-sodium as Repr. 2, H361d. In particular, RMS argues that dose dependency is important to their conclusion. However, for 'unossified odontoid bone' they ignore the fact that in this study the control group frequency per litter (9.2%) was much lower than the HCD minimum (14-36%), and that even the low dose group, whilst having a statistically significantly increased frequency than control at 12.8%, was also below the HCD minimum. In the case of 'centrum not ossified' the same applies to the control group which had a frequency below the HCD (71.0% vs 74 - 95.5%), whilst the low and mid dose had frequencies within the HCD (79.2 and 87.0 respectively). It also seems unreasonable to place such emphasis on a common variant that occurs at such a high frequency in untreated foetuses. According to the guidance on CLP, retardation of ossification in association with maternal toxicity is not sufficient to justify classification.

In this study maternal toxicity, in terms of reduced food consumption and reduced bodyweight, was observed at all three dose levels of metam-sodium. Therefore, no conclusion can be made for this study as to whether developmental toxicity was observed at a dose level without maternal toxicity.

OECD 414 in the Rabbit – Hellwig and Hildebrand, 1987

In this study, female rabbits were exposed to metam-sodium at 0, 10, 30, and 100 mg/kg/day on days 6-18 of gestation. Food consumption was reduced in the high dose group but not in the low and mid dose groups. Bodyweight gain was reduced in the high dose group and also in the mid dose group at several time periods but without any impact on the terminal bodyweight. There was a marked and statistically significant reduction in the number of live foetuses at the mid and high dose levels and a corresponding dose related increasing trend in the number of post-implantation losses. The number of post-implantation losses was statistically significantly increased at the mid and high dose levels but not at the low dose. The RMS considers that the evident trend includes the low dose group (3.7, 8, 12*, 55** respectively). However, they do not consider the historical control incidence of post-implantation loss of the testing laboratory, which was not provided in the study report, and so it is not possible to conclude whether the control frequency was abnormally low or that the low dose did indeed show a non-statistically significant increase of concern. Furthermore, the frequency of pre-implantation losses in the control group was double that in the low dose group (17.59% vs 8.74%), and therefore the total frequency of pre- and post-implantation losses was 27% greater in the control than in the low dose group (21.29% vs 16.74%) respectively.

It should also be noted that in the rabbit study of Hodge (1993) the maternal NOAEL was only 5 mg/kg/day, which is lower than all three dose levels used in this study, so the possibility of maternal toxicity occurring at the low dose level (10 mg/kg/day) in this study cannot be excluded.

In terms of skeletal effects, the RMS concludes "…*there were no statistically significant differences between the treated groups and the controls with respect to anomalies,*

variations and retardations. Most of the findings noted are found to about the same degree in treated and untreated animals and/or in the historical control data." However, the RMS further concludes, "…*it is confirmed that skeletal anomalies are dose-dependently increased at 30 mg/kg b.w./d and above*,…", although this statement is difficult to comprehend because in their table B.6.6.2.2/04-3 they show skeletal anomalies as being zero in all four dose groups. It may be that they refer specifically to the incidence of 'Sternum asymmetrical sternebrae' where the frequency was 1/81, 2/85, 7/73, 6/48 in the four dose groups respectively. These correspond to 1.2, 2.4, 9.6, 12.5% respectively, where the HCD range is 0.6 – 6%. It seems to be an extremely precautionary interpretation of the data to identify a trend when the low dose group incidence was less than half of the upper HCD range. Also, it is difficult to imagine a toxicity mode of action that specifically induces asymmetrical sternebrae but has no impact on the frequency of skeletal anomalies, variations, and retardations. Based on this interpretation of the data, and the observation of single incidences of anomalies at the top dose, the RMS proposes that the NOAEL for developmental toxicity is <10 mg/kg/day and that this supports the proposal to classify as Repr.2 H361d. However, the data do not support this interpretation, rather the NOAEL for developmental toxicity should be correctly identified as 10 mg/kg/day, the same as selected for maternal toxicity.

OECD 414 in the Rabbit – Hodge, 1993

In this study, female rabbits were exposed to metam-sodium at 0, 5, 20, and 60 mg/kg/day on days 8-20 of gestation. Food consumption was markedly reduced at the two higher dose levels but not in the low dose group, although the RMS considers that bodyweight gain during the dosing period was clearly reduced at all three dose levels. However, there appears to be an error in Table 6.6.2.2/03-3 because the mean bodyweight of the control group increased by ~1 kilo from day 19 to day 20 (4136 to 5154g), and then reduces to 4243g on day 23. The data indicate that maternal bodyweight during gestation was reduced only in the mid and upper dose groups. In terms of major defects, the applicant regards the small number of observed defects as being spontaneous in origin. However, the RMS considers that, whilst not entirely convincing evidence for teratogenicity, the study data, in combination with the other studies, indicates a potential for a relationship between metam-sodium and craniofacial defects.

The applicant concluded that the NOAEL for maternal toxicity was 5 mg/kg/bw and that the NOAEL for developmental toxicity was 20 mg/kg/day based on increased fetotoxicity at 60 mg/kg/bw. However, the RMS, whilst agreeing with the NOAEL for maternal toxicity, concluded that the NOAEL for developmental toxicity should also be 5 mg/kg/bw based on an increased incidence of foetuses with minor skeletal defects or variants at 20 mg/kg/day. However, this seems to be a rather selective opinion because the OECD 414 informs us that the litter is the unit of analysis and the proportion of litters showing minor skeletal defects was 20/20, 15/16, 16/17, 8/9 in the control to high dose groups respectively. So, on a litter basis there was no increase in the proportion of litters with such defects. The same observation may be made for skeletal variants. The RMS includes the observation of a trend

towards skeletal ossification delays as a part justification, together with the incidence of meningocele and cleft palate, to support a proposal to classify metam-sodium as Repr.2, H361d. However, this contradicts the CLP guidance document that indicates that ossification delays in association with maternal toxicity do not support classification. Furthermore, it is logical to expect a trend in ossification delays because there was a trend of increasing maternal toxicity with dose.

Skeletal Ossification, Meningocele, Spina Bifida, and Maternal Food Consumption

In the four developmental toxicity studies a very low incidence of meningocele and or spina bifida was observed, but often above the HCD because these abnormalities are rare in the strains of rat and rabbit used in the studies. However, whilst placing significant emphasis on these observations in support of their proposal to classify metam-sodium, the RMS also states "*However, the occurrence of meningocele and spina bifida, which are rare anomalies in the strain of rabbits used in this study, may indicate a substance-related effect, particularly as meningoceles were also found to a certain degree in a prenatal toxicity study in rats.* ***It is known from the literature that spina bifida and meningocele may have the same etiology and sometimes occur together. Retardation of ossification may be one contributing factor for the development of these lesions***." The last sentence of the quote above indicates that the RMS considers the retardation of ossification may be a factor for these lesions. Retardation of ossification is a common result of maternal toxicity, reduced maternal and foetal bodyweight, and reduced maternal food consumption. In this case, it seems that these low incidence anomalies can be argued as being secondary to maternal toxicity rather than a direct teratological effect of metam-sodium. Therefore, these observations do not support the proposal to classify metam-sodium as Repr.2 H361d.

It should be noted that in a diet restriction study in the rabbit, Cappon et al., 2005 observed a low incidence of meningocele and omphalocele, and a marked dose related trend in the number of foetuses showing skeletal retardation in ossification (3.9, 4.6, 14.9, 19.3, 12.5, 19.1%) following diet restriction during days 7 – 20 (150, 110, 75, 55, 35, 15 g/day respectively). In another diet restriction study in rabbits (Clark et al., 1986), there was a large increase in the number of malformations observed in the foetuses of rabbits restricted to 15g feed per day on days 6 to 18; specifically relevant to the studies on metam-sodium were sternebral malformations, omphalocele, and craniostenosis. In a dietary restriction study in rats (Fleeman et al., 2005) it was shown that dietary restriction caused an increase in skeletal variations and retarded foetal growth sufficient to induce changes in skeletal development (retardation of ossification), in particular unossified sternebrae, and a dose related trend in the number of membranous ventricular septal defects.

The RMS also noted that anomalies of the eyes (microphthalmia bilateral, anophthalmia), cleft palate, and head (hydrocephaly, exencephaly) were observed at a very low frequency in some of the studies, but only at dose levels where maternal toxicity was evident. Whilst these specific anomalies have not been reported in the very few diet restriction teratology studies that have been published, it is clear that craniofacial defects such as meningocele have been observed following diet restriction. Considering the very low incidence of these

anomalies in the studies on metam-sodium it is reasonable to consider them as either spontaneous in origin or secondary to maternal toxicity.

These dietary restriction studies clearly support the opinion that reduced maternal food consumption in reproductive and developmental toxicity studies is clearly a confounding factor that must be considered in any weight of evidence analysis.

Conclusion

The RMS in its assessment and conclusion, concludes that "*Overall, it would appear that, taking into consideration some uncertainties on the observed developmental effects, and the possible confounder with maternal toxicity, that the classification Repr.2, H361d «Suspected of damaging the unborn child» for the a.s. metam sodium remains justified*." The RMS supports this conclusion with several observations:

1. Consistent effects of ossification delay in a dose-dependent manner;
2. Variants and retardations mostly observed in the presence of maternal toxicity but not always;
3. Rare malformations are found in the presence of maternal toxicity. However, the nature of these findings is somehow consistent, and pertains to craniofacial defects and vertebral column findings;
4. GSH-depletion caused by metam-sodium cannot be used to disregard developmental effects;
5. That some impurities and breakdown products of metam-sodium may contribute to developmental effects.

The first three observations of the RMS can be discounted on the basis that the dose levels that induced maternal toxicity, and the symptoms (clinical signs, etc), that were observed in the studies, was variable between the two studies in rat and the two studies in rabbit. So, it is not sustainable to argue that variants and retardations were sometimes observed in the absence of maternal toxicity. Indeed, the dose levels selected for use in the four studies may be considered to have been excessive in terms of the requirements of OECD 414.

The fact that ossification effects occurred in a dose dependent manner simply reflects the increasing maternal toxicity with dose and is to be expected. The rare malformations that were observed were at a low frequency and have also been observed in diet restriction studies in both the rabbit and the rat. Furthermore, the RMS itself points out that the mechanism of formation of these malformations may be associated with retardation of ossification.

The implication of impurities and break down products is pure speculation and is not based on evidence.

In conclusion, a reasonable and balanced weight of evidence evaluation of the data available for the developmental toxicity potential of metam-sodium, that follows the advice of the CLP guidance document, should arrive at a conclusion that the effects observed in

these studies were secondary to maternal toxicity. There is insufficient evidence to identify a teratogenic mode of action distinct to the known and published secondary modes of action related to maternal toxicity. On this basis, it may be concluded with confidence that there is no justification to propose a classification of Repr.2 H361d for metam-sodium.

References

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ECHA Guidance on the Application of the CLP Criteria Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures. Version 5.0, July 2017

Note. [redacted] is an independent toxicologist working for a regulatory consultancy company. The time expended reviewing the relevant documents and preparing this opinion were compensated by the applicant via EPPA. However, the opinion expressed in this document is mine alone and is based on my toxicological experience and knowledge. The applicant and EPPA had no influence on the expression of my opinion.