UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

009030

JAN 14 1992

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Metam Sodium - Review of Additional Data Submitted by the Registrant in Support of the Developmental Toxicity Studies in Rats and Rabbits with Metam Sodium

Tox Chem No.: 780
HED Project Nos: 2-0341 & 2-0656

FROM: Yiannakis M. Ioannou, Ph.D., Section Head
Review Section I, Toxicology Branch II
Health Effects Division (H7509C)

TO: Susan Lewis, PM21
Herbicide-Fungicide Branch
Registration Division (H7505C)

THRU: Marcia van Gemert, Ph.D., Chief
Toxicology Branch II
Health Effects Division (H7509C)

Registrant: BASF Corporation, Research Triangle Park, N.C.

Action Requested: Review additional data submitted by the registrant in support of the developmental toxicity studies in rats and rabbits with Metam Sodium

Toxicology Branch II has recently completed the evaluation of the additional data submitted by the Metam Sodium Task Force concerning the developmental toxicity studies in rats and rabbits with Metam Sodium. The Agency's evaluation of the additional data and final recommendations appear in the attached reports prepared by Dr. K. E. Whitby and Dr. S. C. Dapson. At present, both studies remain classified as Supplementary Data based on the fact that most of the major issues raised by the EPA reviewers in the original review of these studies have not been resolved.

Attachments:

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WASHINGTON, D.C. 20460

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OFFICE OF
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SUBSTANCES

MEMORANDUM

SUBJECT: Addendum to MRID No. 41577101: Report on the Study of the Prenatal Toxicity of Metam Sodium in Rats After Oral Administration (Gavage)

TO: Susan Lewis PM 21
Registration Division (H7508C)

FROM: K. E. Whitby, Ph.D. 11/1/91
Section, II
Toxicology Branch II/(HED) (H7509C)

THRU: K. Clark Swentzel 10/1/91
Section Head
Toxicology Branch II/(HED) (H7509C)

and

Marcia van Gemert, Ph.D. 11/4/91
Chief, Toxicology Branch II/(HED) (H7509C)

The Metam Sodium Task Force C/O ICI Americas Inc., has submitted 86-5 formatted rebuttals to the "Data Gaps" for guideline number §83-3(a) which were identified in the Data Call-In Notice, Reregistration Phase 4 for the active ingredient Metam Sodium.

The Metam Sodium Task Force has considered the Agency's review of the study and submits the following information to address the deficiencies noted in the DER:

1. Purity of test Substance

Response of the Task Force

The purity of the test substance was stated to be 42.2% w/w, on p. 14, section 3.1 of the report as well as in the supplement to the report where the analyses of the dosing solutions were presented. In the preparation of the dosing solutions no correction for the a.i. content was made. In the analyses of the dosing solutions the 42.2% formulation was used as the comparison standard. The analytical certificate, with English translation is attached.

EPA Response

The supplemental information or peer review worksheet of the California Department of Food and Agriculture Medical Toxicology Branch for the Metam Sodium review (dated 9/25/89) states in section III (Nature of Supplemental Information) that the rebuttal asserts 'Please note that Metam Sodium (sodium methyldithiocarbamate [anhydrous]) is synthesized in aqueous solution, then diluted with water to the desired concentration to meet the label guarantee since the technical material is never isolated."

The current response from the Task Force does not shed any further light upon the issue. More specifically, it is not clear if the 100 mg/100 mL solution contained 100 mg of the active ingredient/100 mL of solution or if the dosing solution contained 100 mg of the 42.2% aqueous stock solution.

Furthermore, the statement in the current rebuttal to EPA - the dosing solution was not adjusted for the 42.2% purity, implies that the theoretical concentrations were not achieved and the actual concentrations were:

Theoretical Concentration (mg/kg)	Actual Concentration (mg/kg)
0	0
10	4.22
40	16.88
120	50.64

This would also imply that the LOEL would be in the range of 4.2 mg/kg and the meningoceles observed in the main study were observed at approximately 50.6 mg/kg.

This point remains to be clarified. I was unable to locate the analytical certificate with English translation.

2. Analytical Data

Response of the Task Force

Due to the relatively short period of stability of the dosing solutions, analysis needed to be done on the day of preparation. The first samples sent for such analysis were not actually analyzed till 4 or 6 days later and thus resulted in low values. This was corrected by the reserve sample, which had been stored in sealed containers at -80°C, being submitted and found to be at the correct concentration when analyzed on receipt by the analytical laboratory. Consequently the results of the first samples were not used. All subsequent samples were analyzed on the day of preparation. All dosing formulations were prepared on the day of dosing. Explanation of this to the analytical laboratory is

enclosed, with an English translation.

EPA Response

The rebuttal indicates that the reason the analytical laboratory data from January 29 and February 12, 1986 could not be used, was due to stability problems. The rebuttal further indicates that the problems were corrected by using the reserve sample. It is assumed that this is a reserve sample of a dosing solution which was prepared 1/29/86. If this is true, how could the date of **preparation** have been 2/24/86 as indicated on p. 333 (which was after the last animal was sacrificed). The same sample numbers were used for 1/29/86 and 2/24/86. There are data for three samples with the number 2. One was prepared January 29, 1986, the other two were prepared February 24, 1986.

The ambiguity regarding the analytical samples remains unresolved.

3. Raw Data From Dose Range Finding Study

Response of the Task Force

The detailed results from the range finding study were contained in the study protocol submitted in the supplement to the report. This is all the information that is available to the Task Force at this time. Since the toxicity elicited at the top dose in the range finding study justifies the selection of the dose levels used in the main study, we question the need for the submission of raw data from the range finding study.

EPA Response

The raw data were requested to make comparisons of dose levels and effects which overlap in the dose range finding and main studies. Although the summary of the range finding study states that 25 animals were assigned to each group, it is not apparent how many were pregnant in each group. The summary also indicates that the number of dead implantations shows a statistically significant increase. The abbreviated table in the protocol only gives the total number of dead implants (early, late, intermediate and total) and the percentage of dead implantations. This information does not indicate the % of postimplantation loss (an evaluation on the litter basis), or which groups were significant.

4. Raw Data for Daily Clinical Exam of Dams

Response of the Task Force

Copies of the individual dams clinical observation record are attached.

EPA Response

What has been provided are German hand written photocopies of fair quality. Not all entries and headings have been translated.

5. Raw Data for Dam Necropsy

Response of the Task Force

Copies of the individual dams necropsy data are contained on the attached data sheets.

EPA Response

The page preceding the dam clinical observation sheets states that the section contains individual dam clinical observations and necropsy records (annotated with English translation where necessary). Since translations were provided only "where necessary" I can not ascertain whether the entries pertain to clinical observations or to necropsy.

6. Fetal Sex Ratio

Response of the Task Force

The sex distribution (total number of pups by sex and overall) in each group was contained in Table 16, in the top row of the table as the number of males and the number of females. The ratios (male:female) derived from these figures are: Group 1, 1.03:1, Group 2, 0.7:1, Group 3, 0.8:1, Group 4, 1.02:1.

EPA Response

This is satisfactory.

7. Presentation of Fetal Litter Data

Response of the Task Force

Female 91, fetuses 3 and 5 showed meningocoele. This was confirmed at the visceral examination to which they were assigned. Their fetal weights were not exceptional both within the litter and within the group. From Table 042 it can be seen that this litter contained 18 fetuses, and one early resorption. From Table B011 and B012, it can be seen that these fetuses were of normal weight (91/3 had a weight of 3.22 grams and 91/5 had a weight of 3.10 grams, the lowest weight in the litter being 3.00 grams and the highest 3.66 grams. The mean fetal weight in the litter was 3.26 grams.) Both fetuses were males, and the only additional observation was pelvic dilatation in fetus 91/3.

The manner in which the conducting laboratory undertake their procedures is such that the fetuses are individually traceable in the raw data. Specifically, the fetuses in a litter are held

together by a string in the order that they occur within the uterus and their assignment to either visceral or skeletal examination. They are then retained in this manner till the final visceral or skeletal examination is completed. The observations for any fetus would then be consistently recorded against the position number within the litter that the fetus occupied. This correlation is possible in the raw data. The manner in which the fetal data is presented in the report does not make it possible to make these correlations. However, in this particular instance, only two fetuses out of 261 showed this anomaly and hence any correlations that could be made in terms of fetal weight etc., would not be of any significance in the overall interpretation of this study.

EPA Response

This point was raised because the reviewer could not determine the fate of the 2 fetuses with meningocele. Table 20 (cesarean section anomalies) indicates that meningocele was observed in the heads of 2 fetuses at the highest dose tested. Table 25 (anomalies found during evisceration) lists bilateral microphthalmia in the head of one fetus at the highest dose tested. Table 30 (organ anomalies from the Barrow/Taylor) does not indicate any anomalies. If the meningocele finding was confirmed at the visceral examination, why do the tables not reflect the finding?

The only findings that the reviewer was able to find in the tabulated data that could even remotely be considered related to neural tube defects in the region of the head, were found in the skeletal data. The incidences of these observations pertaining to frontal, parietal or interparietal bones did not match the incidence of the reported meningoceles.

8. Split of Fetuses for Skeletal and Soft-Tissue Examination

Response of the Task Force

The practice of taking 1/3 of the litter for visceral examination was a common practice at the time of the conduct of the study. This does not however, affect the ability to detect neural tube changes in all of the fetuses. The defect seen in this study was detected as an external change and not initially as a visceral evaluation. All fetuses in this study were given a detailed external examination and neural tube defects of this type are normally present as external anomalies. However, should an occult defect of this nature occur, this could be detected by either visceral or skeletal evaluations. As the skeletal elements investing the neural tube and brain are dependent on the normal formation of these neural organs for their development, any alteration in the formation of these nervous tissues result in the disruption of normal skeletal formation in that region. Thus neural tube defects result in at least a widening of the vertebral

neural arches, if not more severe disruption. Meningocoeles would alter the position and formation of the dermal bones in the cranium, particularly the parietal and interparietal bones. They would be detected in all phases of the examinations carried out in the study.

The two fetuses showing meningocoele, as stated above, were subjected to visceral examination and the observations were confirmed in this examination. The absence of the type of defect in the skeleton that would have been caused by such anomalies is evidence that no other similar anomalies occurred in this study. The fact that the compound under test had caused a much larger number of meningocoeles in the range finding study at higher doses would have sensitized the personnel involved in the fetal evaluations to be very much more aware of the lesion and thus increased the likelihood of detection of such lesions in this study.

Hence, overall it is clear that the full incidence of meningocoele in this study has been described and is confined to two fetuses in one litter at the highest dose tested. Thus the evaluation of a greater proportion of fetuses for visceral anomalies than was actually done in this study would not have provided any better potential for detection of neural tube anomalies.

EPA Response

Page 2 of the Data Evaluation Record states "Given that the administration of this test substance appears to result in meningocele (in two species - rat and rabbit) it is the opinion of this reviewer that examination of 2/3's of each litter for soft tissue changes as directed in the guidelines would have provided a better assessment of the potential for neural defects." The statement does **not** say that evaluation of 2/3's of each litter for soft tissue changes would provide a better assessment of neural **tube** defects.

This statement was not directed toward detection of neural tube defects. This statement was in reference to internal defects of the soft tissue; the organization or irregularities in the development of tissues or organs contained within the cranium. Considering the manner in which the fetuses were split it is uncertain if an adequate assessment of the potential for alterations in soft tissue development of this nature were made. Because it was demonstrated that tissues in and of the head may be sensitive to an effect, concern regarding how the litters were split for examination could not be alleviated since the fetuses have already been processed.

By examining only 1/3 of the litter for soft tissue changes, the probability of finding soft tissue changes of this nature were decreased. Although, by some standards and in certain situations

the "practice of taking 1/3 of the litter for visceral examination was a common practice at the time of conduct of the study", the study was conducted in 1986. The EPA Subdivision F Pesticide Assessment Guidelines referenced in the DER were written in 1982 (the 1984 revised edition did not modify this aspect). The Food and Drug Administration also provided the same recommendation for safety assessment of direct food and color additives in their 1982 "Red Book". The recommendation to examine 1/3 to one half of each litter for skeletal anomalies and the remaining part of each litter for soft tissue anomalies was based upon sound scientific judgement and was not capricious. It is agreed that in some situations splitting the litter so that 2/3 are examined for skeletal anomalies may be appropriate or may not impair the identification of potential hazard. However, given the current scenario it is not the opinion of this reviewer, based upon the available data, that this was such an instance.

9. Bacterial Content of Water

Response of the Task Force

The analytical results presented in the supplement relate to the entire site on which the laboratory is situated. The specific analyses relative to the laboratory are those listed under building Z470, where the bacterial content was found to be negligible at worst. The instances of high bacterial content were not relevant to this study. The analyses are conducted on a batch basis by the local water authorities and for ease of reporting present the results for the entire site.

EPA Response

This is satisfactory.

10. Number of Animals for the Study

One hundred male rats in the weight range of 200-250 grams were obtained from the breeder on 7th January, 1986. These formed a stock group of stud males used over a number of studies.

Two batches of females 55-65 days old, were obtained from the same source for use in teratology studies. Two hundred twenty-five females were received on the 14th January 1986 and 226 females on the 21st January 1986. These were mated with the stock males and the first 90 females found to have mated from the first batch and 10 from the second batch were used on this study. The remaining females that had mated were used on other concurrent studies. The mating procedure was to house four females with each male overnight and to examine vaginal smears for the presence of sperm the following morning.

EPA Response

The strain and source of the males was not stated.

11. Lack of NOEL

Response of the Task Force

It is assumed that this is based on the interpretation of the pre and post implantation loss in the study that is discussed at greater length on p. 15 of the EPA review.

As stated in the report it is our contention that 10 mg/kg/day is the NOEL for developmental toxicity.

Selected Cesarean Data

Dose mg/kg/day	0	10	40	120
Parameter				
Corpora Lutea/Dam	15.21	15.63	14.50	15.05
Implantations/Dam	13.75	13.71	12.46	13.95
Live Fetuses/Dam	12.63	11.75	11.54	11.86
Preimplantation Loss(%)	10.37	10.20	15.24	7.37
Postimplantation Loss(%)	7.28	17.89*	6.66	14.79*

The reviewer states that at the middle dose (40 mg/kg/day) there was no increase in postimplantation loss, that the number of live fetuses was similar to controls, but that there was an increase in preimplantation loss. However, at the lower dose of 10 mg/kg/day and the higher dose of 120 mg/kg/day there was an increase in postimplantation loss. The proportionate increase in postimplantation loss in the lowest dose was higher than that in the highest dose, which together with the lack of increase in postimplantation loss in the middle dose demonstrates the absence of a dose response for postimplantation loss.

Further, had the postimplantation loss in the 10 mg/kg/day been caused by the test material and the increase to 40 mg/kg/day have exacerbated this effect to an increase in preimplantation loss, i.e. a more severe effect, then it would be logical to expect this effect to be further enhanced by increasing the dose further to 120 mg/kg/day at which dose even greater preimplantation losses would have been expected. This clearly did not occur, preimplantation loss being similar to control values at 120 mg/kg/day. Further, had the effect at the middle dose (40 mg/kg/day) been due to the test compound, then an increase in postimplantation loss as well as preimplantation loss would have been expected. This again did not occur.

As the Salewski staining procedure was used on empty uterine horns, all postimplantation losses would have been detected. Hence the preimplantation loss figures would not include any undetected

postimplantation losses. Therefore the increase in preimplantation loss at 40 mg/kg could not have been caused by exposure to the test substance.

An updated table of historical control values, covering the period 1984-1989, is attached herewith and shows that the value for postimplantation loss at 10 mg/kg /day is only just outside the historical control range.

When the performance of the individual dams in the study are considered with the control groups of contemporary studies, as in the attached figure, it can be seen that the 10 mg/kg/day group is not significantly different to other control groups. In addition it is also clear that the high postimplantation loss figure in this group is heavily biased by the contribution of three females, (females 34, 46, and 50), as the calculation was a litter based one, i.e. the percentage value for each litter was first calculated and then the group mean derived from these litter mean values. Litters such as that from female 50, with only one implant which happens to be dead would contribute a 100% value to the mean and thus heavily bias it.

If the three suspect females are excluded for the calculations of the group mean for postimplantation loss, the value obtained is 10.7, which is similar to the concurrent control and well with in the historical control range.

Thus viewed overall the increase in postimplantation loss at the 10 mg/kg/day level is not a consequence of treatment due to the lack of the lack of a coherent dose response relationship with the higher doses. It is concluded that 40 mg/kg is the NOEL for postimplantation losses in this study.

EPA Response

This reviewer did not state that the increased preimplantation loss at the mid dose was a result of treatment. The statement made regarding increased preimplantation loss, which was only observed in the 40 mg/kg group, was intended to demonstrate that the 40 mg/kg group was aberrant. This group in all likelihood was the "outlier" not the 10 mg/kg group. The discussion of data on p. 15 of the DER was included to support this opinion. The 40 mg/kg group is not consistent with the concurrent control or other treatment groups. More specifically the mean numbers of corpora lutea, implantations, and resorptions per dam, are decreased in the 40 mg/kg group, while the % of live fetuses/dam and preimplantation loss are increased. An aberrant group would not be likely to fit in a dose response relationship.

This reviewer is still of the opinion that the postimplantation loss at the lowest dose tested is a treatment related finding for

the following reasons:

1. The values for postimplantation loss and % live fetuses/dam in the 10 mg/kg group exceed the concurrent and available historical control data (see table providing comparisons):

a. The historical control data submitted with the rebuttal reports a mean postimplantation loss of 7.9 (SD±11.3). The mean value is within the range of the concurrent study control but not the 10 mg/kg group. The SD for this parameter is rather high.

b. If the % of dead implants provided for the dose range finding concurrent control are used as a rough estimate of postimplantation loss, it is also within the range of the concurrent control, but not the 10 mg/kg group.

c. The historical control data for the Wistar rat which was submitted with the study is within the general range of the concurrent control, but not the 10 mg/kg group.

2. The rebuttal suggests that 3 animals in the 10 mg/kg group be omitted from the calculations since they heavily bias the post implantation loss.

a. Omitting females 34, 46 and 50 N = 21 would yield a mean postimplantation loss of **10.71**. If female 34 (14 implants 6 of which were non viable = 42.86% postimplantation loss for this female) is added to the group mean, the mean group postimplantation loss would be **12.17** (N = 22). If female 46 (13 implants 8 of which are non viable = 61.54% postimplantation loss for this female) is added to the group mean, the mean group postimplantation loss would be **14.32** (N = 23). When all females in the group are included the value is **17.89**.

b. Although the 3 dams may influence the mean, omitting them still leaves a mean which exceeds the concurrent control and the 40 mg/kg group. This would lend further credence to the notion that the effects observed in the low dose may be related to treatment and that a dose response relationship was not established due to some unknown factor within the 40 mg/kg group which apparently affected the implantation data.

c. Omitting female 50 from the calculation may be valid since this female had only one implant which was nonviable (postimplantation loss for this female = 100%). However, this would yield a mean % postimplantation loss of 14.32 for the 10 mg/kg group, which is still 96.7% above the concurrent control or 81.3% above the historical control mean provided in the rebuttal.

d. On November 1, 1991, I asked Hugh M. Pettigrew, Ph.D. a statistician with the Science Analysis and Coordination Branch of HED (H7509C), whether omission of dam # 50 from statistical analyses would still result in a significar increase in postimplantation loss. Dr. Pettigrew approached this question by two different analyses.

(1) A Student's t-test comparing 2 means was performed: the control group = 7.28 and the 10 mg/kg group = [illegible] omitting dam # 50). The test determined t [illegible]6. For this data to be significant at the 0.[illegible] level, t would have to equal 1.68. However, since the data may be considered to have been biased by throwing out a data point, this may be considered as significant.

(2) A 2 X 2 comparison was made between the control and 10 mg/kg group for the number of dams with either 0 dead implantations or > 0 dead implantations. This was found to be highly significant.

	0 dead	> 0 dead	
0 mg/kg	15	8	23
10 mg/kg	6	18	24
	21	26	47

The p value for the Fisher Exact Test when comparing 8/23 vs 18/24 was 0.0062.

3. Furthermore, in the DER the observation was made that there were significant increases in the % fetuses/litter with anomalies, variations, and retardations at the 40 mg/kg level, which were dose-related (except for anomalies). There were significant increases in the % fetuses/litter with variations and retardations at the 120 mg/kg level which were dose-related. There were dose-related increases in thoracic vertebrae bodies dumbbell shaped with unchanged cartilage, metacarpals and metatarsals that were incompletely ossified (unilaterally) with cartilage present, and generalized retardation. The presence of a dose response relationship for these observations also indicates that there may not be a NOEL.

In summary based upon the available information which has been presented to date, it is not possible for this reviewer to definitively rule out the possibility of a treatment related effect

at the lowest dose tested. Therefore, I must conclude that the NOEL is somewhere below 10 mg/kg.

Comparison of Control and Metam Sodium Treated
Treated Dams and Fetal Wastage

Endpoint	10 mg/kg	40 mg/kg	Concurrent Control	Wistar Rat				Vit A	D-R Finding Study Control	Breeder Data	Rebuttal Historical Control Data
				CMC	oil	inhalation	water				
# Pregnant	24	24	24	47	24	42	23	25	-	608	420
Mean # C.L.	15.63	14.5	15.21	-	-	-	-	14.55	-	-	15.7
Mean # Impl/Dam	13.71	12.46	13.75	13.81	13.67	13.69	14.04	11.91	-	-	14.3
Mean # Live Fetuses/Dam	11.75	11.54	12.63	-	-	-	-	11.09	-	-	13.2
% Live Fetuses/Dam	82.11	93.34	92.72	90.03	92.60	93.82	90.94	92.41	-	-	-
Resorptions/Dam	1.96	0.92	1.13	-	-	-	-	0.82	-	-	1.1
Preimplantation Loss	10.20	15.24	10.37	-	-	-	-	-	-	9.79	9.1
Postimplantation Loss	17.89 or 14.32	6.66	7.28	9.97	7.4	6.18	9.06	7.59	*	10.16	7.9

* available data indicate that the % of dead implantations is 7.99.

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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SUBSTANCES

MEMORANDUM

SUBJECT: Additional data submitted in support of the developmental toxicity study in rabbits with Metam-Sodium (MRID No.403309-01).
EPA ID # 039003-010182, EPA Barcode D170894, MRID No. none, EPA Pesticide Chemical Code 039003, Toxicology Chemical No.780, HED Project No. 2-0341 and 2-0656.

TO: Christine Rice/Tom Myers, PM 52
SRRD (H7508W)

FROM : Stephen C. Dapson, Ph.D. Stephen C. Dapson 10/30/91
Senior Pharmacologist, Review Section I
Toxicology Branch II/HED (H7509C)

THRU : Yiannakis M. Ioannou, Ph.D., D.A.B.T. J M Ioannou 10/31/91
Section Head, Review Section I
and
Marcia van Gemert, Ph.D.
Branch Chief, Toxicology Branch II Marcia van Gemert 11/4/91
Health Effects Division (H7509C)

Registrant : Metam Sodium Task Force
c/o ICI AMERICAS INC.
Agricultural Products
Wilmington, Delaware 19897

Action Requested : Review of additional data submitted in support of the developmental toxicity study in rabbits with Metam-Sodium (MRID No.403309-01).

Recommendations : Toxicology Branch II has reviewed the additional data submitted in support of the study "Report on the Study of the Prenatal Toxicity of Metam-Sodium (Aqueous Solution) in Rabbits After Oral Administration (Gavage)" and determined that the study cannot be upgraded at this time due to the fact that the study deficiencies have not been resolved to the Agency's satisfaction.

BACKGROUND:

The Toxicology Branch II reviewed the study: Report on the Study of the Prenatal Toxicity of Metam-Sodium (aqueous solution) in rabbits after Oral Administration (gavage) (MRID No. 403309-01, Project No. 38R0232/8579, July 15, 1987). The following are the conclusions from the study: Dose Levels tested: 10, 30, and 100 mg/kg by gavage from gestation days 6 through 18 with a 42.2% aqueous solution of metam-sodium in Himalayan rabbits.

Maternal Toxicity NOEL = 10 mg/kg/day
Maternal Toxicity LOEL = 30 mg/kg/day

Maternal Toxicity consisted of reduced body weight gains, reduced food consumption, increased number of dead implantations and reduced numbers of fetuses, and increased post-implantation loss in either mid or high dose group or both.

Developmental Toxicity was apparent in the mid and high dose in the form of increased number of dead implantations and reduced numbers of fetuses, and increased post-implantation loss; however the Developmental Toxicity NOEL and LOEL cannot be determined with available data; additional information is required.

Core Classification: Core-Supplementary Data

This study does not satisfy the guideline requirements (§ 83-3) for a teratology study in rabbits. Additional data are required; if these data are supplied and found acceptable to the Agency the study may be upgraded.

The registrant responded as follows:

The Agency review of these data is in general agreement with that provided in the study report. However, the reviewer felt that due to seven (7) study deficiencies a developmental toxicity NOEL and LOEL could not be determined at the time of the review. The reviewer stated that the study could be upgraded if the deficiencies are corrected and that a developmental toxicity NOEL would then be determined.

Based on its review of the information provided regarding the foetal skeletal examination techniques, the Task Force believes that a developmental toxicity NOEL of 10 mg/kg/bodyweight/day can be determined. Each of the deficiences as listed in the Agency review are addressed below. The Task Force believes that these data will upgrade this teratogenicity study in rabbits to acceptable.

The following is the Agency response to the Company Response:

1. EPA Comment:

Purity was apparently only 42.2% a.i., must be clarified.

Company Response:

Metam sodium was available as a 42.2% w/w solution as this is the maximum solubility of the substance in water at ambient temperatures. A copy of the analytical certificate and an English translation is attached. In the preparation of the dosing solutions no correction for the a.i. content was made. In the analyses of the dosing solutions the 42.2% formulation was used as the comparison standard. The analytical supplement, with English translation, is attached.

EPA Response:

According to the registrant's response, "no corrections for the a.i. content was made"; this would indicate that the actual doses the animals received were 42.2% of the stated dose as follows.

Stated target dose	Actual dose
10 mg/kg	4.22 mg/kg
30 mg/kg	12.66 mg/kg
100 mg/kg	42.2 mg/kg

Thus, the actual dose levels used in the rabbit developmental toxicity study are considered to be 4.2, 12.7, and 42.2 mg/kg/day and not 10, 30, and 100 mg/kg/day as stated in the study report. This deficiency is considered resolved.

2. EPA Comment:

Analysis of test compound and concentrations were provided in German only.

Company Response:

An English translation is attached.

EPA Response:

The analytical methodology was provided separately from the actual analytical report. The "english translation" for the "concentration control analysis" provided indicates that the target concentration was met in samples tested. The methodology supplied indicates that "Metam-Fluid is the name for a series of water soluble concentrates with 380 to 560 g/l Metam sodium in water." The study states on page 4 that "Metam-Fluid 510 g/l" was used, and on page 26 that "The percentage of active ingredient was 42.2% or 517.3 g/l." Based on the provided information, this deficiency is considered resolved.

3. **EPA Comment:**

Artificial insemination procedure was not provided.

Company Response:

Conventional artificial insemination procedures, as commonly practiced, were utilized by the conducting laboratory. The procedure is covered by a Laboratory Standard Operating Procedure SOP. This SOP is available only in German at this time.

EPA Response:

Since the SOP is only available in German at this time, the technique used cannot be verified and compared to other known techniques. This is therefore still considered a study deficiency.

4. **EPA Comment:**

Reference to techniques [used in the study] was not provided.

Company Response:

Citation of these references is attached.

EPA Response:

The list is acceptable to the Agency; this deficiency is thus resolved.

5. **EPA Comment:**

Pilot study was not provided. Must be provided.

Company Response:

The detailed results of the pilot study were contained in the study protocol, which was in the Supplement attached to the report. Pages 4 and 5 of the EPA review reproduce results (dead implantions, live fetuses, uterus weights, body weights) from the pilot study. These data, in addition to the fact that the highest dose tested was a toxic dose, justify the selection of the dose levels for the main study quite adequately.

EPA Response:

The complete pilot study must be provided, including both group mean and individual animal data. The Agency agrees that the pilot study indicated that the dose levels tested appear adequate; however, the study is still needed to assess possible patterns of effects that are related to the primary teratology study.

6 A. **EPA Comment:**

The fetal skeletal examinations were carried out by a method that has not been validated by the EPA and further, according to the investigators, if the radiograph was not clear, a separate technique was used. All fetuses must be examined by a validated staining technique.

Company Response:

The EPA guidelines, as with the OECD guidelines, do not stipulate a staining technique for the skeletal examination. They both state that the skeletons should be prepared and evaluated. (Section 83-3 of the Pesticide Assessment Guidelines states: "for rabbits, each foetus should be examined by careful dissection for visceral anomalies and then examined for skeletal anomalies." Similarly for rats it states "...should be prepared and examined for skeletal anomalies..."

The X-ray technique used by the laboratory is a standard technique that has been used by a number of laboratories and is based on the publications of Stertz et al. 1975, 1987 and Nothdurft 1977. Submissions using this techique have been found acceptable to the EPA in the past. In addition, the CAL-EPA has determined this study acceptable. In our view the X-ray technique has adequately evaluated the skeletons on this study and we believe it should be acceptable to the EPA.

References:

Stertz et al. 1975. Routine X-ray pictures of the skeletons of rabbit foetuses of day 31 post-conceptionem versus 3-dimensional preparations. Teratology 12, 335.

Stertz et al. 1987. Teratologic studies on the Himalayan rabbit: new aspects of thalidomide induced teratogenesis. Arch. Toxicol. 60, 376-381.

Nothdurft et al. 1977. Routine radiography of skeletons of 31 day old rabbit foetuses. In "Methods in prenatal toxicology" Eds. Neubert et al. Springer-Verlag, 1977.

EPA Response:

The Agency does not accept the x-ray technique for examination of skeletal anomalies of the fetuses and still requires that all fetuses be examined by a validated staining technique. This study will not be upgraded until all fetuses are examined for skeletal anomalies by a validated staining technique. A new study will be required if fetuses from this study were not preserved as per CFR 40 § 160 (GLP's). Thus, this deficiency is not resolved.

6 B. **EPA Comment:**

...if the radiograph was not clear a separate technique was used.

Company Response:

This procedure was necessitated by the time lag between the taking of the X-ray photograph and the availability of the radiograph for evaluation. Where the skeletal evaluation was not clear due to the positioning of the foetus on the radiograph, the litter concerned was stained with alizarin and the skeleton examined. In this study this procedure was used for 7 litters.

EPA Response:

According to the company response, the investigators examined 7 litters by the alternate technique, this was not apparent in the report. This must be resolved.

7. **EPA Comment:**

Clinical observations and gross pathology observations were not supplied as summary tables and/or as raw data, this must be provided.

Company Response:

Copies of the clinical observations and gross pathology for individual dams are attached.

EPA Response:

Data were provided as hand-written data sheets in German (with some english translation) from experimental notebooks, with some of the information not legible. The way the data are presented, make it impossible to draw any conclusions on clinical observations and gross pathology. This deficiency remains unresolved

Conclusions:

The study "Report on the Study of the Prenatal Toxicity of Metam-Sodium (Aqueous Solution) in Rabbits After Oral Administration (Gavage)" cannot be upgraded at this time as the study deficiencies have not been resolved to the Agency's satisfaction.