External Peer Review

U. S. Environmental Protection Agency

A Comparative Chronic Toxicity Study of Methyl n-Propyl Ketone, Methyl n-Butyl Ketone, and Hexane by Ingestion

Final Report

Final Compilation of Reviewer Comments And Responses to Charge Questions

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Integrated Risk Information System (IRIS) Program
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The ORISE IRIS Technical Assistance Team has neither altered nor edited these comments for grammatical or other errors.

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CHARGE TO EXTERNAL PEER REVIEWERS
FOR THE
2-HEXANONE FINAL REPORT

Subject: Peer review of report entitled, "A Comparative Chronic Toxicity Study of Methyl n-Propyl Ketone, Methyl n-Butyl Ketone, and Hexane by Ingestion", written by J.L. O'Donoghue, W.J. Krasavage, C.J. Terhaar (Eastman Kodak) in 1978.

The U.S. Environmental Protection Agency's (EPA) National Center for Environmental Assessment (NCEA) is currently developing a human health assessment of the chemical 2-hexanone (methyl n-butyl ketone; CASRN 591-78-6). The report from Eastman Kodak entitled, "A Comparative Chronic Toxicity Study of Methyl n-Propyl Ketone, Methyl n-Butyl Ketone, and Hexane by Ingestion" (1978), is proposed as principal support for the development of one or more toxicity values for 2-hexanone, but this report has not been subjected to a formal peer review process. Such a peer review process is important to establishing the appropriateness, validity, and robustness of the study design, conduct, and interpretation of findings of the reported investigation.

As indicated by the report title, the study described is that of a combined repeated-exposure and neurotoxicity study in CD/COBS (SD) rats. In this 13-month study, male CD/COBS (SD) rats (10/group) were exposed to drinking water containing 0, 0.25, 0.5, or 1.0% (0, 143, 266, or 560 mg/kg-day) 2-hexanone (96% pure, containing 3.2% MiBK and 0.7% unknown contaminants). Body weight and neurological examinations were performed weekly.

Please limit your review and response to the following charge questions to data relating to methyl n-butyl ketone. The results on methyl n-propyl ketone and hexane outlined in the report are beyond the scope this charge and thus review of such data is not required.

Charge Questions

- Based on your knowledge of toxicological protocols, please comment on the experimental design of this investigation. Do you see any significant issues with the test system or test article employed, inhalation exposure equipment and monitoring of atmosphere, endpoints recorded, terminal procedures, statistical analyses, and quality assurance?

- Are there physiological/toxicological endpoints that should have been assessed that were not part of the investigation?

- Please comment on the strength, credibility, and relevance of the toxicological results. Were the individual animal data correctly summarized and interpreted?

- Is the discussion on methyl n-butyl ketone (page 13) supported by the data? Are the neuropathology observations on methyl n-butyl ketone (page 8-11) supported by the presented data? Were there critical results or issues that were not discussed or addressed in the results or discussion? Were there any contradictory statements or observations in the study regarding methyl n-butyl ketone?

- In your opinion, was this investigation properly planned, conducted, and reported? Are there any procedures, observations or analyses that would have added to the quality of this investigation?

External Peer Review

REVIEWER RESPONSES

QUESTION 1

Based on your knowledge of toxicological protocols, please comment on the experimental design of this investigation. Do you see any significant issues with the test system or test article employed, inhalation exposure equipment and monitoring of atmosphere, endpoints recorded, terminal procedures, statistical analyses, and quality assurance?

Response from Lucio Costa

The study, conducted in 1978, is a chronic (13 weeks) study of the oral (in drinking water) toxicity of MPK, in which MnBK was used as a positive control. N-hexane was also used as a positive control, however, because of poor water solubility, exposure could not be quantified. The concentrations of MnBK used were 0.25, 0.5 and 1.0%. Water consumption is not indicated but was apparently measured, as dose levels of 143, 266 and 560 mg/kg/day of MnBK were estimated.

Response from Darol Dodd

The experimental design of the study was appropriate for being a narrowly focused study. Examples of the study being narrowly focused follow.

- One sex of rats were used
- Weekly neurological examinations
- Perfusion of 50% of animals with glutaraldehyde at necropsy
- Special embedding and tissue staining procedures of CNS and PNS tissues for histopathological evaluation
- No feed consumption, hematology, clinical chemistry, urinalysis, or ophthalmologic measurements
- Reduced number of organs weighed at necropsy

Clearly, the study was designed as a chronic neurotoxicity study, though light microscopic examination of several non neuronal tissues was also performed in high dose and control animals (Table 6).

Using this study design, interpretation of findings would be limited for non neuronal effects, such as reproductive, immunologic, endocrinologic, hematologic, etc.

The study predates today's common use of Good Laboratory Practice standards (GLPs), a requirement for toxicity testing of chemicals and chemical compounds when data are to be submitted to a regulatory agency. Thus, laboratory operations and procedures for endpoints recorded may not have been adequately validated, and additional experimental design procedures, such as stability assessment of test agent in vehicle (MnBK in water) may not have been performed. For example, it's unclear if freshly prepared dosing solutions were administered every other day or stored dosing solutions were administered every other day. Personal opinion: The reputation of Eastman Kodak's toxicology laboratory to provide high quality data is and has been excellent (assuming the study was conducted in this laboratory).

Response from Marion Ehrich

The test compound (methyl n-butyl ketone, MnBK) was administered in the drinking water with consumption measured every other day so mg/kg dosages could be calculated. These are reported in Table 1. The text suggests that these are daily doses, but does not specifically say this either in the text or as a legend for Table 1. The test substance was analyzed by gas chromatography at 3.2% MnBK with 0.7% unknown contaminants. Although unstated, it is assumed that the 1.0%, 0.5% and 0.25% MnBK concentrations in water were based on the information gathered from GC analysis. Therefore, there appears to be good likelihood that the amount of MnBK that the animals received is provided with reasonable accuracy in the document.

Animal housing data are not provided (temperature, humidity, hours of light). This is not unusual for reporting done in the 1970's and does not detract from the worth of the study. The method of providing the drinking water was also not specifically stated, although it can be assumed that this was through sipper bottles, since consumption was measured. The methods used for animal sacrifice (carbon dioxide inhalation and anesthesia with perfusion) are appropriate, and many tissues were collected and examined.

Statistical analysis was only done on organ weights. The method used (ANOVA followed by Duncan's procedure) was appropriate.

QUESTION 2

Are there physiological/toxicological endpoints that should have been assessed that were not part of the investigation?

Response from Lucio Costa

Measured end-points included body weight, clinical non neural and neurological signs of toxicity, gross pathological changes and histopathological changes, with an emphasis on the nervous system. No information was provided on water consumption and food consumption (which would be relevant in view of the significant effect on body weight). No determination of hematological functions was carried out. The number of animals is relatively small, as some measurements were only carried out in 4-5 animals.

Response from Darol Dodd

The objective of the study was not clearly defined by the investigators, but a likely assumption is that it was designed as a chronic neurotoxicity study. The key endpoints for this type of study include behavioral assessments and histopathology of neuronal tissue. Neurochemical measurements in neuronal tissue, scheduled-controlled operant behavior assessments, and sensory evoked potential recordings are also important, but they are generally performed to propose or support specific neurotoxic effects or mode-of-action investigations. This study included weekly neurological examinations and histopathological examination of CNS and PNS tissues. However, details of the weekly neurological examinations are not reported. It does appear from information provided in the results section (page 7), that some measures of a functional observational battery (FOB) were performed, but not motor activity. There is no description of how paralysis was determined. Thus, the study design is weak for assessing behavioral function, especially quantitatively.

The report did not provide drinking water data. It also did not indicate if there were alterations in water consumption in MnBK-treated animals, possibly due to taste aversion. Since food consumption was not measured, the decrease in body weight gain (Figure 3) is difficult to interpret as a primary or a secondary effect.

Response from Marion Ehrich

The investigators looked at appropriate endpoints (body weight, organ weights, clinical signs, gross and histopathology). They reported non-neural clinical signs on an individual animal basis. Methods for assessing neurobehavioral and neuropathological changes, however, were different and less rigorous in the 1970's than they are now (see comments in Question 3 below).

QUESTION 3

Please comment on the strength, credibility, and relevance of the toxicological results. Were the individual animal data correctly summarized and interpreted?

Response from Lucio Costa

Results are presented in a somewhat incomplete fashion. For example, statistical analysis is not presented in Tables, and there is no indication of whether it was performed for some end-points (e.g. Tables 6, 7, 8). No illustration of typical toxic effects (e.g. swollen axons) is presented.

Response from Darol Dodd

Results of the study clearly indicate that MnBK is neurotoxic, and that the predominant effect is in peripheral nerves. This information has been supported by numerous scientific studies for more than 30 years. Further, a clear dose-response relationship was established for the endpoint of concern (neuropathology). Thus, this study is credible and relevant. However, its strength for measuring potential effects of MnBK beyond the nervous system was weak.

Response from Marion Ehrich

This investigation was completed in 1978, which was before a standardized Functional Observational Battery was recommended for use in neurotoxicity studies (Moser et al., Fund. Appl. Toxicol. 1988). Therefore, the neural clinical signs are purely descriptive, with animals scored only as 'slight," "moderate," or "severe" for neural clinical signs. Some statements are vague (e.g., The clinical course was highly variable with improvements in the clinical weakness being very common.). There didn't appear to be a descriptor or any quantitative evaluation of the improvements.

On the neuropathology, descriptors are again vague. What is meant when saying "swollen axons were common"? or "myelin ovoids were frequently found along with degenerating axons"? The neuropathological data provided in Table 8 is only a ratio of the number affected compared to the total number observed, so there is no indication of the severity of changes, such as number of giant (swollen) axons, number of degenerating axons, etc. This makes the reader unsure of how different 0.5% MnBK animals were from animals given higher doses, as "giant axons were generally few in number but were found in all animals." Generally the adjectives used in the text to describe the neuropathology are vague ("few," "numerous", "infrequent", "minimal").

In this study MnBK was used to provide a positive control (neurotoxic substance) for an investigation of its analog, methyl n-propyl ketone. The investigators did, however, summarize by stating correctly that rats given MnBK showed dose-related deficits.

QUESTION 4

Is the discussion on methyl n-butyl ketone (page 13) supported by the data? Are the neuropathology observations on methyl n-butyl ketone (page 8-11) supported by the presented data? Were there critical results or issues that were not discussed or addressed in the results or discussion? Were there any contradictory statements or observations in the study regarding methyl n-butyl ketone?

Response from Lucio Costa

The neuropathology observations (p. 8-11) appear to be supported by the data presented in Table 8. The conclusion (p. 13) indicates that at all dose levels MnBK causes neurotoxicity. At the low dose, neuropathological effects were seen, even in the absence of clinical neurological signs. All three dose levels caused significant decreases in body weight. Based on the results, the dose of 143 mg/kg/day appears to be a LOAEL for MnBK in this study.

Response from Darol Dodd

The discussion of neuropathology appears to be supported by the results. However, data were not available in the report to calculate dose (mg/kg/day). Though a dose-response relationship was observed for neuropathy, the last sentence in the discussion is not supported with any other data. Thus, a statement by the authors that the low dose of MnBK was close to a threshold is not justified.

A summary of the neuropathology associated with MnBK treatment (pages 8-11) was informative and adequate, but individual animal pathology data were not available to confirm incidence data (Table 8).

The authors remained focused on the neuropathologic effects of MnBK. Yet, Tables 6 and 7 provided histopathologic results for several non neuronal tissues and tumors. No discussion was provided on potential liver effects (relative liver weight was increased compared to control groups at all MnBK dose levels) or testicular effects (increase in relative testes weight and observation of bilateral focal hypospermatogenesis).

Response from Marion Ehrich

The authors of this report give 143 mg/kg as the dose received by consumption of 0.25% MnBK in drinking water (the lowest concentration tested). Since no references are provided at the point that statement is provided in the document, a reader cannot know that the neurotoxicity of MnBK was reported at a level lower than that reported previously. Based on the dose-related decrease in deficits, it could be said that 143 mg/kg may approach a threshold dose, but such a statement would be strengthened by results with a lower dose.

It would help to have summarized data other than Table 8 (which only gave ratios of number affected over number dosed) to support the statement on page 13 that peripheral nerves seemed to be more affected. How much more notable are 10/10 animals with peripheral nerve than 8/10 animals with brain giant axons? Or if 8/10 animals have peripheral nerve giant axons but 7/10 have spinal cord giant axons? Tabulating the "slight" and "severe" would have been helpful among those affected would have been helpful, as it qualitative differences are difficult to assess through text alone.

QUESTION 5

In your opinion, was this investigation properly planned, conducted, and reported? Are there any procedures, observations or analyses that would have added to the quality of this investigation?

Response from Lucio Costa

The study may have been fully acceptable in 1978, but would probably not be judged satisfactory if done today. There is a lack of details on methodology (including statistical analysis), and on reported findings (water and food consumption). Standard hematological tests were not carried out. However, the study's primary goal was apparently that of determining whether MPK shared the same neurotoxic properties of MnBK, and from this point of view, it would seem that MPK is not neurotoxic. On the other hand, there is clear demonstration (based on effects on body weight and on neuropathology) that MnBK causes significant adverse effects at the dose level of 143 mg/kg/day.

Response from Darol Dodd

Designed as a chronic neurotoxicity study, this investigation was properly planned, conducted, and reported. More details should have been provided to better understand methods and procedures. Neurobehavioral assessments appear to be largely subjective during weekly clinical examinations. This is a weakness in the study. Since the study predates routine use of GLPs, this is another weakness in the study. Any interpretation of non neuronal findings (tissue histopathology) would be equivocal, because the study was not designed to assess other toxicity endpoints (e.g., reproductive, immunologic, endocrinologic, hematologic, etc.).

Response from Marion Ehrich

This investigation was conducted appropriately according to procedures of the time. The investigators were very thorough, especially with reporting body weights, individual non-neural clinical signs, and histological lesions in the multitude of organs they collected. Comments above indicate that a study done today would have numerical and statistically analyzable data for clinical neurotoxicity, and grading systems for the neuropathology.