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DRAFT

MUT/08/9

COMMITTEE ON MUTAGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT (COM)

GENERIC PAPERS ON HYPOTHERMIA AND HYPERTHERMIA AND INDUCTION OF MICRONUCLEI IN RODENT BONE MARROW MN TESTS

Introduction

1. Members will wish to see the appended generic papers on hypothermia and hyperthermia as potential modes of action for induction of micronuclei in rodent bone marrow MN tests (Annex 1).

Hypothermia

2. Tweats et al (Mutation Res, 627,78-91, 2006) reviewed the evidence for hypothermia as a mode of induction of MN in mouse bone marrow tests using chlorpromazine, reserpine, E-5824 (an atypical antipsychotic pharmaceutical), phenol and Covance compound 4. All of these compounds induced hypothermia in mice of sufficient duration to cover dosing and sampling times in bone marrow MN tests. The effect of chemical induced hypothermia could be reversed by keeping animals in environments with elevated temperatures (data were reported for chlorpromazine, reserpine, E-5824 and Covance compound 4). It is noted that rats were resistant to the hypothermic effects of these chemicals and no MN induction was seen in rodents.

3. The data for phenol differs from the other chemicals reviewed in that a clear *in-vitro* mutagenic effect inducing chromosomal damage and micronuclei probably via an oxidative DNA damage mechanism. In addition the reversal of phenol induced hypothermia has not been reported in mouse bone marrow MN tests.

4. An interesting *in-vitro* study of hypothermia in CHL cells indicated possible effects of hypothermia on spindle function as one possible mode of action for hypothermic induction on MN. (Asanami et al J of Tox Sci, 26, 323-326, 2001)

Hyperthermia

5. The artificial induction of elevated body temperatures in mice has been reported to result in the induction of increased MN frequency in bone marrow PCEs. (Asanami and Shimono K Mutat Res, 390, 79-83, 1997).

6. Oxymorphone (an opioid analgesic) induced hyperthermia in rats was associated with an increase in the frequency of MNPCEs in bone marrow. The effect could be abolished by pre-treatment with sodium salicylate to reduce oxymorphone-induced elevated body temperature. (Shuey D et al, Tox

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Sci, 95, 369-375, 2007). A similar but more variable effect of oxymorphone was also reported in mice.

Comment

7. The data support the observation that chemical induced hypothermia in mice and hyperthermia in rats and mice may be potential modes of induction of MN in bone marrow. Experimental evidence needed to support hypothermia or hyperthermia as a mode of action for an unknown chemical would include a time course showing the association between core body temperature and MN induction and evidence for reversibility of the chemical induced MN formation by adjusting core body temperature. The mechanism by which hypothermia can result in the induction of MN in mice is unclear. Thus single doses of ethanol can induce significant hypothermia in susceptible rats and mice but no evidence for *in vivo* mutagenicity was reported in rodents (COM statement 00/S5, December 2000). The assessment of hypothermic induction of MN for a specific chemical also requires evaluation for evidence regarding other modes of genotoxicity. A clear negative *in vitro* package of genotoxicity tests would rule out other modes of genotoxicity when deriving conclusions regarding the role of hypothermia in any observed *in vivo* MN formation. Evidence for positive *in vitro* genotoxicity would suggest other potential modes of genotoxic action which need to be taken into account in the overall assessment.

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[Addict Biol.](#) 2003 Dec;8(4):419-27. [Links](#)

Age- and sex-related differences in alcohol and nicotine effects in C57BL/6J mice.

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The present studies were aimed to identify possible age- and/or sex-related differences in the effects of alcohol and nicotine. Study 1 examined age-related differences in alcohol and nicotine effects. Adolescent and adult C57BL/6J male mice were injected with alcohol or nicotine. Results indicated that alcohol and nicotine induced hypothermia and reduced locomotor activity in both adolescent and adult mice. In both dependent variables, adults were more affected than adolescents that received the same alcohol or nicotine dose. Study 2 examined possible sex-related differences in the effects of these drugs. Results replicated the aged-related differences revealed in Study 1 after alcohol or nicotine administration in male mice. No sex-related differences were observed in alcohol effects. However, young animals of both sexes and adult females appeared to be more resistant to nicotine effects. In both studies, blood alcohol concentrations and cotinine plasma concentrations were assessed. These results suggest that young C57BL/6J mice are more resistant to both alcohol and nicotine effects. In addition, adult females may be more resistant to acute nicotine effects on temperature and locomotion.

[Psychopharmacology \(Berl\)](#). 2002 Jul;162(3):313-22. Epub 2002 May

14.  [FULL-TEXT ARTICLE](#) [Links](#)

Initial sensitivity, tolerance and cross-tolerance to allopregnanolone- and ethanol-induced hypothermia in selected mouse lines.

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RATIONALE: Acute ethanol administration induces hypothermia in genetically susceptible animals. Tolerance to this effect may develop with repeated administration. Allopregnanolone is an endogenously produced neuroactive steroid that acts at the GABA-A receptor. We postulated that allopregnanolone would induce hypothermia, and that lines of mice selectively

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bred for high (COLD-1 and COLD-2) or low (HOT-1 and HOT-2) sensitivity to ethanol's hypothermic effects would also be differentially sensitive to allopregnanolone-induced hypothermia. We also postulated that tolerance would develop to these two drugs by similar mechanisms, such that tolerance to one would impart cross-tolerance to the other. **OBJECTIVES:** To assess sensitivity, tolerance and cross-tolerance to allopregnanolone's and ethanol's hypothermic effects in HOT-1 and 2, and COLD-1 and 2 mice. **METHODS:** Mice were administered one of several doses of allopregnanolone each day, for 4 days, and initial sensitivity and tolerance to allopregnanolone-induced hypothermia were assessed. On day 5, ethanol was administered to assess cross-tolerance. In a separate experiment, COLD-1 and 2 mice were made tolerant to ethanol's hypothermic effects, and challenged with allopregnanolone to assess cross-tolerance. **RESULTS:** COLD mice exhibited greater initial sensitivity to the hypothermic effect of allopregnanolone, as compared to HOT mice. Tolerance to allopregnanolone-induced hypothermia was greater in COLD mice than in HOT mice, but only COLD-1 mice showed cross-tolerance to ethanol. Both replicate lines of COLD mice developed tolerance following repeated administration of ethanol, but only COLD-2 mice showed cross-tolerance to allopregnanolone. **CONCLUSIONS:** These results demonstrate shared genetic influence over allopregnanolone and ethanol's initial hypothermic effects. They also suggest genotype-dependent differences in the mechanisms for tolerance to these two compounds.