

Search Descriptions for Searches Related to the Registration of
Butoxyethanol CAS# 111-76-2
Searches Conducted Sept. 27th and Oct. 1st, 2013

By: Megan Goucher and Kimberly Bateman, MLIS
Toxicology & Environmental Research and Consulting
The Dow Chemical Company

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Report Summary

Purpose:

The purpose of this report is a literature search for the new hazard information for "Butoxyethanol" CASRN 111-76-2 induced liver carcinogenicity in rodents and to search for information on other chemicals that may work through a similar mode of actions and summarize the literature search process for future reference.

Summary:

The searches were conducted over a two day period. An attempt has been made to remove duplicates, so the final search result numbers do not reflect the exact count produced by each database.

Databases searched:

CAPLUS (Chemical Abstracts Plus); PubMed/MEDLINE; TOXNET (Including ChemIDPlus, DART, GeneTOX, EMIC and TOXLINE); Web of Science

Terms Used:

Various combinations of the following terms were used in the searches.

Butoxyethanol, 111-76-2, MOA or Mode of Action, Hemolysis, Alkoxyacetic Acid, Hemolysis, Liver Carcinogenicity, Genotoxicity, Kupffer cells.

CAplus

CAplus (Chemical Abstracts Plus)

Years of Coverage: 1907 to present with additional materials from as far back as 1808

CAplus was searched on Oct. 1, 2013 by Kimberly Bateman MLIS, Librarian, Toxicology & Environmental Research and Consulting (TERC) of The Dow Chemical Company.

Terms used in the search:

111-76-2/rn and ((MOA or mode of action) or Hemolysis or (Alkoxyacetic (w) Acid) or (Liver (w) Carcinogenicity) or Genotoxicity or (Kupffer (w) cells))

Limits applied:

Exclude patents, Publication years: 2000 - Present

The search resulted in 30 unique hits.

1. Allen, D. G., et al. (2004). "Prediction of Rodent Carcinogenesis: An Evaluation of Prechronic Liver Lesions as Forecasters of Liver Tumors in NTP Carcinogenicity Studies." Toxicol Pathol **32**(4): 393-401 CODEN: TOPADD; ISSN: 0192-6233 PUBLISHER: Taylor & Francis.

The National Toxicol. Program (NTP) developed the chronic 2-yr bioassay as a mechanism for predicting the carcinogenic potential of chems. in humans. The cost and duration of these studies has limited their use to small nos. of selected chems. Many different short-term methods aimed at increasing predictive accuracy and the no. of chems. evaluated have been developed in attempts to successfully correlate their results with evidence of carcinogenicity (or lack of carcinogenicity) are assessed. Using NTP studies, the effectiveness of correlating prechronic liver lesions with liver cancer encompassing multiple studies using mice (83 compds.) and rats (87 compds.). These lesions include hepatocellular necrosis, hepatocellular hypertrophy, hepatocellular cytomegaly, bile duct hyperplasia, and hepatocellular degeneration, along with increased liver wt. These results indicate that pooling 3 of these prechronic data points (hepatocellular necrosis, hepatocellular hypertrophy, and hepatocellular cytomegaly) can be very predictive of carcinogenicity in the 2-yr study ($p < 0.05$). The inclusion of increased liver wt. as an endpoint in the pool of data points increases the no. of rodent liver carcinogens that are successfully predicted ($p < 0.05$), but also results in the prediction of increased nos. of noncarcinogenic chems. as carcinogens. The use of multiple prechronic study endpoints provides supplementary information that enhances the predictivity of identifying chems. with carcinogenic potential. OS.CITING REF COUNT: 42 THERE ARE 42 CAPLUS RECORDS THAT CITE THIS RECORD (43 CITINGS)

2. Anadon, A., et al. (2011). "Scientific opinion on Flavouring Group Evaluation 10, Revision 2 (FGE.10Rev2): aliphatic primary and secondary saturated and unsaturated alcohols, aldehydes, acetals, carboxylic acids and esters containing an additional oxygenated

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functional group and lactones from chemical groups 9, 13 and 30." EFSA Journal **9(7)**: 2164.

The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids of the European Food Safety Authority was requested to evaluate 61 flavoring substances in the Flavouring Group Evaluation 10, Revision 2, using the Procedure in Commission Regulation (EC) No 1565/2000. None of the substances were considered to have genotoxic potential. The substances were evaluated through a stepwise approach (the Procedure) that integrates information on structure-activity relationships, intake from current uses, toxicol. threshold of concern, and available data on metab. and toxicity. The Panel concluded that the 61 substances do not give rise to safety concerns at their levels of dietary intake, estd. on the basis of the MSDI approach. Besides the safety assessment of these flavoring substances, the specifications for the materials of commerce have also been considered. For four substances, information on compn. of mixt. and/or stereoisomerism has not been specified sufficiently.

3. Corley, R. A., et al. (2005). "Determination of age and gender differences in biochemical processes affecting the disposition of 2-butoxyethanol and its metabolites in mice and rats to improve PBPK modeling." Toxicol Lett **156(1)**: 127-161.

2-Butoxyethanol (BE) is the most widely used glycol ether solvent. BEs major metabolite, butoxyacetic acid (BAA), causes ***hemolysis*** with significant species differences in sensitivity. Several PBPK models have been developed over the past two decades to describe the disposition of BE and BAA in male rats and humans to refine health risk assessments. More recent efforts by Lee et al. [1998] to describe the kinetics of BE and BAA in the National Toxicol. Program (NTP) chronic inhalation studies required the use of several assumptions to extrapolate model parameters from earlier PBPK models developed for young male rats to include female F344 and both sexes of B6C3F1 mice and the effects of aging. To replace these assumptions, studies were conducted to det. the impact of age, gender and species on the metab. of BE, and the tissue partitioning, renal acid transport and plasma protein binding of BAA. In the current study, the Lee et al. PBPK model was updated and expanded to include the further metab. of BAA and the salivary excretion of BE and BAA which may contribute to the forestomach irritation obsd. in mice in the NTP study. The revised model predicted that peak blood concns. of BAA achieved following 6 h inhalation exposures are greatest in young adult female rats at concns. up to 300 ppm. This is not the case predicted for old (.gtoreq.18 mo) animals, where peak blood concns. of BAA in male and female mice were similar to or greater than female rats. The revised model serves as a quant. tool for integrating an extensive pharmacokinetic and mechanistic database into a format that can readily be used to compare internal dosimetry across dose, route of exposure and species. OS.CITING REF COUNT: 3 THERE ARE 3 CAPLUS RECORDS THAT CITE THIS RECORD (3 CITINGS)

4. Garnier, R. C. S. h. F.-W. C. d. p. p. P. c. F. (2010). "EGBE kidney toxicity and ethyl acetate reproductive toxicity." Archives des Maladies Professionnelles et de l'Environnement **71(4)**: 703.

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A review. A brief expert opinion on the renal toxicity of ethylene glycol monobutyl ether (EGBE, 2-butoxyethanol) and on reproductive toxicity of ethanol formed during metabolic hydrolysis from Et acetate in humans is provided. The available data confirm the nephrotoxicity of EGBE, although the mechanisms are not yet clear and may involve tubular pptn. of Hb from ***hemolysis*** induced by butoxyacetic acid (EGBE metabolite). The ethanol reproductive toxicity after Et acetate solvent exposure appears unlikely when proper occupational exposure limits are obsd.

5. Gehlhaus, M. W., III, et al. (2011). "Approaches to cancer assessment in EPA's Integrated Risk Information System." Toxicol Appl Pharmacol **254**(2): 170-180.

A review. The U.S. Environmental Protection Agency's (EPA) Integrated Risk Information System (IRIS) Program develops assessments of health effects that may result from chronic exposure to chems. in the environment. The IRIS database contains more than 540 assessments. When supported by available data, IRIS assessments provide quant. analyses of carcinogenic effects. Since publication of EPA's 2005 Guidelines for Carcinogen Risk Assessment, IRIS cancer assessments have implemented new approaches recommended in these guidelines and expanded the use of complex scientific methods to perform quant. dose-response assessments. Two case studies of the application of the ***mode*** of ***action*** framework from the 2005 Cancer Guidelines are presented in this paper. The first is a case study of 1,2,3-trichloropropane, as an example of a chem. with a mutagenic mode of carcinogenic action thus warranting the application of age-dependent adjustment factors for early-life exposure; the second is a case study of ethylene glycol monobutyl ether, as an example of a chem. with a carcinogenic action consistent with a nonlinear extrapolation approach. The use of physiol. based pharmacokinetic (PBPK) modeling to quantify interindividual variability and account for human parameter uncertainty as part of a quant. cancer assessment is illustrated using a case study involving probabilistic PBPK modeling for dichloromethane. We also discuss statistical issues in assessing trends and model fit for tumor dose-response data, anal. of the combined risk from multiple types of tumors, and application of life-table methods for using human data to derive cancer risk ests. These issues reflect the complexity and challenges faced in assessing the carcinogenic risks from exposure to environmental chems., and provide a view of the current trends in IRIS carcinogenicity risk assessment. OS.CITING REF COUNT: 1 THERE ARE 1 CAPLUS RECORDS THAT CITE THIS RECORD (1 CITINGS)

6. Ghanayem, B. I., et al. (2001). "Hemolytic anemia, thrombosis, and infarction in male and female F344 rats following gavage exposure to 2-butoxyethanol." Experimental and Toxicologic Pathology **53**(2-3): 97-105.

2-Butoxyethanol (BE; ethylene glycol monobutyl ether) is used extensively in the manuf. of a wide range of domestic and industrial products which may result in human exposure and toxicity. BE causes severe hemolytic anemia in male and female rats and mice. In a recent report, female F344 rats exposed to 500 ppm BE by inhalation and sacrificed moribund on day 4 of treatment exhibited disseminated thrombosis assocd. with infarction in several organs. In contrast, no such lesions were obsd. in male rats similarly exposed to BE. Addnl. studies were therefore undertaken to compare the

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effects of BE in rats of both sexes. Rats received 250 mg BE/kg/day by gavage for 1, 2, or 3 days and were sacrificed 24 or 48 h after the last dose. Control rats received 5 mL/kg water. Progressive time-dependent hemolytic anemia - macrocytic, hypochromic, and regenerative - was obsd. in both sexes of rats exposed to BE. Addnl., BE caused significant morphol. changes in erythrocytes, first obsd. 24 h after a single dose, including stomatocytosis, macrocytosis with moderate rouleaux formation, and spherocytosis. These morphol. changes became progressively more severe as BE dosing continued and included the occasional occurrence of schistocytes and ghost cells, rouleaux formation in rats of both sexes, and an increased no. of red blood cells with micronuclei in female rats. Overall, the progression of hemolytic anemia and morphol. changes as a function of the no. of days of exposure varied with gender and suggested a faster onset of ***hemolysis*** in female rats. The range of BE-related histopathol. changes noted in both sexes was comparable; however, while these lesions were obsd. in female rats following a single dose, similar effects were first obsd. in males after 3 consecutive days of exposure to BE. Pathol. changes involved disseminated thrombosis in the lungs, nasal submucosa, eyes, liver, heart, bones, and teeth, with evidence of infarction in the heart, eyes, teeth, and bones. Hemoglobinuric nephrosis and splenic extramedullary hematopoiesis were also noted. An apparent correlation between the severity of hemolytic anemia and subsequent disseminated thrombosis in BE-treated rats is proposed. Thrombosis may be related to intravascular ***hemolysis***, which could be triggered by procoagulant release and(or) alterations in erythrocyte morphol., as well as increased rigidity. OS.CITING REF COUNT: 14 THERE ARE 14 CAPLUS RECORDS THAT CITE THIS RECORD (14 CITINGS)

7. Ghanayem, B. I., et al. (2000). "Comparison of the acute hematotoxicity of 2-butoxyethanol in male and female F344 rats." Human & Experimental Toxicology **19**(3): 185-192.

The administration of 2-butoxyethanol (BE) to rodents causes acute hemolytic anemia, and metabolic activation of BE to butoxyacetic acid (BAA) is required for the development of this effect. Recent studies have shown that female rats treated with BE exhibit a variety of histopathol. lesions that are absent in males and many of these lesions are attributed to the hemolytic effects of BE. Current studies were designed to compare the acute hematotoxicity of BE in male and female F344 rats. Rats were treated with 250 mg BE/kg or water (control; 5 mL/kg) by gavage. At 4, 8, or 24 h after dosing, rats were anesthetized, blood was collected by cardiac puncture, and various blood parameters were measured. BE resulted in a time-dependent swelling of erythrocytes as evidenced by an early increase in hematocrit (Hct) and mean cell vol. (MCV) in male rats. In contrast, increased Hct in female rats did not accompany an increase in MCV. It is likely that ***hemolysis*** was so severe at 4 h that Hct exhibited a decline in female rats at that time point. Subsequently, red blood cell (RBCs), Hb concn. (Hgb), and Hct declined as ***hemolysis*** progressed. However, the onset of BE-induced ***hemolysis*** was faster in female compared to male rats. These effects were also assocd. with a significant increase in the spleen wt. to body wt. ratio. Blood smears were also prepd. and morphol. changes evaluated by light microscopy included stomatocytosis, spherocytosis, and schistocytosis. Furthermore, aggregation of RBCs in female rats as evidenced by increased formation of rouleaux was obsd. at 24 h after BE

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administration. These effects were obsd. earlier and more frequently in female rats. No differences in the sensitivity of RBCs obtained from male and female rats and exposed to butoxyacetic acid (BAA) in vitro was obsd. as detd. by measuring the packed cell vol. Apparently, female rats are more sensitive to ***hemolysis*** and morphol. alterations of erythrocytes induced by BE during the first 24 h after exposure compared to males. It is likely that the greater sensitivity of female rats to BE effects on RBCs may account for the reported development of thrombosis and tissue infarction in female rats. OS.CITING REF COUNT: 12 THERE ARE 12 CAPLUS RECORDS THAT CITE THIS RECORD (12 CITINGS)

8. Gift, J. S. C. S. N. C. f. E. A. U. S. E. P. A. R. T. P. N. C. U. S. A. (2005). "U.S. EPA's IRIS assessment of 2-butoxyethanol: the relationship of noncancer to cancer effects." Toxicol Lett **156**(1): 163-178.

U.S. EPA's integrated risk information system (IRIS) assessment of 2-butoxyethanol (EGBE) indicates that the human carcinogenic potential of EGBE cannot be detd. at this time, but that "suggestive evidence" for cancer exists from lab. animal studies (hemangiosarcoma of the liver in male mice and forestomach squamous cell papilloma or carcinoma in female mice and carcinogenesis studies of 2-butoxyethanol (CAS no. ***111-76-2***) in F344/N rats and B6C3F1 mice). Since the last EGBE IRIS assessment, a no. of studies have provided evidence that the carcinogenic effects obsd. in mice are nonlinear in their ***mode*** of ***action*** and may be dependent on threshold events such as EGBE-induced hemolytic effects. EPA is in the process of considering several questions relating to this issue. First, can a plausible ***mode*** of ***action*** be detd. for the two types of tumors obsd. in mice. Second, are the mechanisms involved applicable to humans. If so, should the ***mode*** of ***action*** be considered to result in a linear or nonlinear dose-response. These questions will be addressed within the context of the agency's new cancer guidelines and with regard to how the answers might affect a revised IRIS assessment for EGBE. OS.CITING REF COUNT: 5 THERE ARE 5 CAPLUS RECORDS THAT CITE THIS RECORD (5 CITINGS)

9. Green, T., et al. (2002). "The development of forestomach tumors in the mouse following exposure to 2-butoxyethanol by inhalation: studies on the mode of action and relevance to humans." Toxicology **180**(3): 257-273.

2-Butoxyethanol, a forestomach carcinogen in mice exposed by inhalation, has been shown to enter the forestomach as a result of grooming and ingestion of material condensed on the skin and fur during exposure. The material entering the stomach concs. in the forestomach region and persists for at least 48 h post-exposure. Mice given single oral doses of either 2-butoxyethanol or 2-butoxyacetic acid, daily for 10 days, developed a marked hyperkeratosis in the forestomach. 2-Butoxyacetic acid was more potent than 2-butoxyethanol, the NOEL for the former being 50 mg/kg and for the latter, 150 mg/kg. Although a dose dependent increase in cell replication was also seen with both chems., the results were confounded by a high labeling rate in the controls. There was no evidence of significant binding of radiolabeled 2-butoxyethanol to proteins in stomach tissues. 2-Butoxyethanol was metabolized in vitro in both mouse and rat forestomach and glandular stomach fractions by alc. dehydrogenases forming 2-

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butoxyacetaldehyde which was rapidly converted by aldehyde dehydrogenases to 2-butoxyacetic acid. There was a marked species difference in alc. dehydrogenase activity between rats and mice with the max. rates up to one order of magnitude greater in mouse than rat. The alc. and aldehyde dehydrogenases were heavily concd. in the stratified squamous epithelium of the forestomach of both rats and mice whereas in the glandular stomach the distribution was more diffuse. In human stomach both enzymes were evenly distributed throughout the epithelial cells of the mucosa. It is concluded that 2-butoxyethanol is ingested following inhalation exposure and concs. in the forestomach where it is metabolized to 2-butoxyacetic acid which causes cellular damage, increased cell replication and hyperkeratosis. These changes are believed to lead to the tumors seen in mice exposed to 2-butoxyethanol for a lifetime. Differences in structure and enzyme distribution between the rodent and human stomach suggest that the responses seen in the mouse are unlikely to occur in humans. OS.CITING REF COUNT: 9 THERE ARE 9 CAPLUS RECORDS THAT CITE THIS RECORD (9 CITINGS)

10. Green, T., et al. (2005). "The development of forestomach tumours in the mouse following exposure to 2-butoxyethanol by inhalation: studies on the mode of action and relevance to humans." Toxicol Lett **156**(1): 103-105.

Letter and commentary concerning development of forestomach tumors in the mouse following exposure to 2-butoxyethanol by inhalation and studies on the ***mode*** of ***action*** and relevance to humans.

11. Gualtieri, J. F., et al. (2003). "Repeated ingestion of 2-butoxyethanol: case report and literature review." Journal of Toxicology, Clinical Toxicology **41**(1): 57-62 CODEN: JTCTDW; ISSN: 0731-3810 PUBLISHER: Marcel Dekker.

Background: Ethylene glycol monobutyl ether (2-butoxyethanol) is not commonly assocd. with significant human poisoning. Exposures are usually through occupational contact and typically involve inhalation injury. Animal studies report severe ***hemolysis*** occurring in rats and mice. Rare published human cases give varied descriptions of the clin. course assocd. with 2-butoxyethanol poisoning including reports of metabolic acidosis, ethylene glycol prodn., oxaluria, renal failure, and anemia. The authors report a case of 2 sep. ingestions (80-100 g) of a glass cleaner conc. contg. 22% 2-butoxyethanol, and its primary metabolite butoxyacetic acid. Case Report: An 18-yr-old male ingested 360-480 mL of 22% 2-butoxyethanol on 2 sep. occasions. Approx. 10 h after the first ingestion, the patient developed severe CNS depression, metabolic acidosis, hematuria, and mild elevation of hepatic enzymes. He was treated initially with ethanol therapy but continued to deteriorate and was started on hemodialysis. Approx. 10 days after discharge, the patient ingested 480 mL of the same product and received ethanol and hemodialysis within 4 h of ingestion. During his second admission the patient did not develop the delayed severe CNS depression or profound metabolic acidosis. Clin. significant hemolytic anemia, oxaluria, ethylene glycol prodn., and renal failure were not noted in either episode. The patient recovered on both occasions without sequelae. Conclusion: Hemodialysis may be an effective treatment intervention for managing severe acute 2-butoxyethanol intoxication; however, further investigation

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is warranted. OS.CITING REF COUNT: 6 THERE ARE 6 CAPLUS RECORDS THAT CITE THIS RECORD (6 CITINGS)

12. Hughes, K., et al. (2001). "2-butoxyethanol: hazard characterization and exposure-response analysis." Journal of Environmental Science and Health, Part C: Environmental Carcinogenesis & Ecotoxicology Review **C19**(1): 77-104 CODEN: JESHA102 PUBLISHER: Marcel Dekker.

2-Butoxyethanol has been assessed as a Priority Substance under the Canadian Environmental Protection Act. Based primarily on investigations in exptl. animals, the crit. health effects assocd. with exposure to 2-butoxyethanol are alterations in hematol. parameters assocd. with ***hemolysis*** and lesions of the forestomach in mice. A tolerable concn. of 11 mg/m³ has been derived, based upon the benchmark concn. for hematol. effects in rats, quant. taking into account exptl. data on interspecies variations in kinetics and dynamics. A tolerable concn. of 0.04 mg/m³ has also been derived based on the benchmark concn. for hyperplasia of the forestomach in mice. There is less confidence in this value, however, due to the paucity of information on mode of induction and, hence, relevance to humans.

13. Kirkland, D., et al. (2005). "Evaluation of the ability of a battery of three in vitro genotoxicity tests to discriminate rodent carcinogens and non-carcinogens. I. Sensitivity, specificity and relative predictivity." Mutation Research, Genetic Toxicology and Environmental Mutagenesis **584**(1-2): 1-256.

The performance of a battery of three of the most commonly used in vitro ***genotoxicity*** tests, i.e., Ames + mouse lymphoma assay (MLA) + in vitro micronucleus (MN) or chromosomal aberrations (CA) test, was evaluated for its ability to discriminate rodent carcinogens and non-carcinogens, from a large database of over 700 chems. compiled from the CPDB ("Gold"), NTP, IARC and other publications. We re-evaluated many (113 MLA and 30 CA) previously published ***genotoxicity*** results in order to categories the performance of these assays using the response categories we established. The sensitivity of the three-test battery was high. Of the 553 carcinogens for which there were valid ***genotoxicity*** data, 93% of the rodent carcinogens evaluated in at least one assay gave pos. results in at least one of the three tests. Combinations of two and three test systems had greater sensitivity than individual tests resulting in sensitivities of around 90% or more, depending on test combination. Only 19 carcinogens (out of 206 tested in all three tests, considering CA and MN as alternatives) gave consistently neg. results in a full three-test battery. Most were either carcinogenic via a non-genotoxic mechanism (liver enzyme inducers, peroxisome proliferators, hormonal carcinogens) considered not necessarily relevant for humans, or were extremely weak (presumed) genotoxic carcinogens (e.g. N-nitrosodiphenylamine). Two carcinogens (5-chloro-o-toluidine, 1,1,2,2-tetrachloroethane) may have a genotoxic element to their carcinogenicity and may have been expected to produce pos. results somewhere in the battery. We identified 183 chems. that were non-carcinogenic after testing in both male and female rats and mice. There were ***genotoxicity*** data on 177 of these. The specificity of the Ames test was reasonable (73.9%), but all mammalian cell tests had very low specificity (i.e. below 45%), and this declined to extremely low levels in combinations of two and three test systems. When all three

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tests were performed, 75-95% of non-carcinogens gave pos. (i.e. false pos.) results in at least one test in the battery. The extremely low specificity highlights the importance of understanding the mechanism by which ***genotoxicity*** may be induced (whether it is relevant for the whole animal or human) and using wt. of evidence approaches to assess the carcinogenic risk from a pos. ***genotoxicity*** signal. It also highlights deficiencies in the current prediction from and understanding of such in vitro results for the in vivo situation. It may even signal the need for either a reassessment of the conditions and criteria for pos. results (cytotoxicity, soly., etc.) or the development and use of a completely new set of in vitro tests (e.g. mutation in transgenic cell lines, systems with inherent metabolic activity avoiding the use of S9, measurement of genetic changes in more cancer-relevant genes or hotspots of genes, etc.). It was very difficult to assess the performance of the in vitro MN test, particularly in combination with other assays, because the published database for this assay is relatively small at this time. The specificity values for the in vitro MN assay may improve if data from a larger proportion of the known non-carcinogens becomes available, and a larger published database of results with the MN assay is urgently needed if this test is to be appreciated for regulatory use. However, specificity levels of <50% will still be unacceptable. Despite these issues, by adopting a relative predictivity (RP) measure (ratio of real:false results), it was possible to establish that pos. results in all three tests indicate the chem. is greater than three times more likely to be a rodent carcinogen than a non-carcinogen. Likewise, neg. results in all three tests indicate the chem. is greater than two times more likely to be a rodent non-carcinogen than a carcinogen. This RP measure is considered a useful tool for industry to assess the likelihood of a chem. possessing carcinogenic potential from batteries of pos. or neg. results.

OS.CITING REF COUNT: 291 THERE ARE 291 CAPLUS RECORDS THAT CITE THIS RECORD (293 CITINGS)

14. Kirkland, D., et al. (2005). "Evaluation of the ability of a battery of three in vitro genotoxicity tests to discriminate rodent carcinogens and non-carcinogens. I. Sensitivity, specificity and relative predictivity. [Erratum to document cited in CA143:243161]." Mutation Research, Genetic Toxicology and Environmental Mutagenesis **588**(1): 70.

On the title page, the URL of the website address in the open star footnote should read: www.lhasalimited.org/cgx. This is where the appendixes have been posted. OS.CITING REF COUNT: 3 THERE ARE 3 CAPLUS RECORDS THAT CITE THIS RECORD (3 CITINGS)

15. Laifenfeld, D., et al. (2010). "The Role of Hypoxia in 2-Butoxyethanol-Induced Hemangiosarcoma." Toxicological Sciences **113**(1): 254-266.

To understand the mol. mechanisms underlying compd.-induced hemangiosarcomas in mice, and therefore, their human relevance, a systems biol. approach was undertaken using transcriptomics and Causal Network Modeling from mice treated with 2-butoxyethanol (2-BE). 2-BE is a hemolytic agent that induces hemangiosarcomas in mice. The authors hypothesized that the ***hemolysis*** induced by 2-BE would result in local tissue hypoxia, a well-documented trigger for endothelial cell proliferation leading to hemangiosarcoma. Gene expression data from bone marrow (BM), liver, and spleen of mice exposed to a single dose (4 h) or 7 daily doses of 2-BE were used to

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develop a mechanistic model of hemangiosarcoma. The resulting mechanistic model confirms previous work proposing that 2-BE induces macrophage activation and inflammation in the liver. In addn., the model supports local tissue hypoxia in the liver and spleen, coupled with increased erythropoietin signaling and erythropoiesis in the spleen and BM, and suppression of mechanisms that contribute to genomic stability, events that could be contributing factors to hemangiosarcoma formation. Finally, an immunohistochem. method (Hypoxyprobe) demonstrated that tissue hypoxia was present in the spleen and BM. Together, the results of this study identify mol. mechanisms that initiate hemangiosarcoma, a key step in understanding safety concerns that can impact drug decision processes, and identified hypoxia as a possible contributing factor for 2-BE-induced hemangiosarcoma in mice. OS.CITING REF COUNT: 7 THERE ARE 7 CAPLUS RECORDS THAT CITE THIS RECORD (7 CITINGS)

16. Lewis, D. A., et al. (2006). "Hemostatic activation in a chemically induced rat model of severe hemolysis and thrombosis." Thrombosis Research **118**(6): 747-753.

Introduction: In hemolytic diseases such as sickle cell disease and .beta.-thalassemia, the mechanisms of thrombosis are poorly understood, however erythrocyte/endothelium interactions are thought to play an important role. Appropriate animal models would increase our understanding of the pathophysiol. of thrombosis and aid in the development of new therapeutic strategies. We previously reported that rats exposed to 2-butoxyethanol (2-BE) develop ***hemolysis*** and enhanced adherence of erythrocytes to the extracellular matrix, possibly secondary to the recruitment of cellular adhesion mol. at the erythrocyte/endothelium interface. Methods: We exposed rats to 250 mg/kg/day of 2-BE for 4 days, and collected blood for coagulation markers on each day. Results: As previously obsd., erythrocytes dropped precipitously (8.0 to 1.8.times.10⁶/.mu.L in 48 h), and diffuse microvascular thrombosis developed in the heart, lungs, liver, bones and eyes. Prothrombin times, activated partial thromboplastin times, fibrinogen, and antithrombin-III were unchanged between treated and control rats, indicating that hemostasis is largely unperturbed. However the thrombin-antithrombin III levels in the 2-BE treated rats for all days were 3-7 times greater than the control rats. The plasma intercellular adhesion mol.-1 (ICAM-1) levels of 2-BE treated animals were approx. twice that of the controls on days 2 and 3 and 1.5 times the controls on day 4 (P < 0.05). Conclusion: Our findings are consistent with the observations of increased erythrocyte aggregation, increased erythrocyte/endothelium interaction, and increased plasma ICAM-1 levels obsd. in sickle cell disease and .beta.-thalassemia patients. This model may be useful for studying therapeutic agents that disrupt erythrocyte/endothelium interactions. OS.CITING REF COUNT: 5 THERE ARE 5 CAPLUS RECORDS THAT CITE THIS RECORD (5 CITINGS)

17. Lockley, D. J., et al. (2005). "Cutaneous metabolism of glycol ethers." Arch Toxicol **79**(3): 160-168.

The toxicity of glycol ethers is assocd. with their oxidn. to the corresponding aldehyde and ***alkoxyacetic*** ***acid*** by cytosolic alc. dehydrogenase (ADH; EC 1.1.1.1.) and aldehyde dehydrogenase (ALDH; 1.2.1.3). Dermal exposure to these compds. can result in localized or systemic toxicity including skin sensitization and irritancy,

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reproductive, developmental and hemotol. effects. It has previously been shown that skin has the capacity for local metab. of applied chems. Therefore, there is a requirement to consider metab. during dermal absorption of these compds. in risk assessment for humans. Cytosolic fractions were prepd. from rat liver, and whole and dermatomed skin by differential centrifugation. Rat skin cytosolic fractions were also prepd. following multiple dermal exposure to dexamethasone, ethanol or 2-butoxyethanol (2-BE). The rate of ethanol, 2-ethoxyethanol (2-EE), ethylene glycol, 2-phenoxyethanol (2-PE) and 2-BE conversion to ***alkoxyacetic*** ***acid*** by ADH/ALDH in these fractions was continuously monitored by UV spectrophotometry via the conversion of NAD⁺ to NADH at 340 nm. Rates of ADH oxidn. by rat liver cytosol were greatest for ethanol followed by 2-EE > ethylene glycol >2-PE >2-BE. However, the order of metab. changed to 2-BE >2-PE > ethylene glycol >2-EE > ethanol using whole and dermatomed rat skin cytosolic fractions, with approx. twice the specific activity in dermatomed skin cytosol relative to whole rat skin. This suggests that ADH and ALDH are localized in the epidermis that constitutes more of the protein in dermatomed skin than whole skin cytosol. Inhibition of ADH oxidn. in rat liver cytosol by pyrazole was greatest for ethanol followed by 2-EE > ethylene glycol >2-PE >2-BE, but it only inhibited ethanol metab. by 40% in skin cytosol. Disulfiram completely inhibited alc. and glycol ether metab. in the liver and skin cytosolic fractions. Although ADH1, ADH2 and ADH3 are expressed at the protein level in rat liver, only ADH1 and ADH2 are selectively inhibited by pyrazole and they constitute the predominant isoforms that metabolize short-chain alcs. in preference to intermediate chain-length alcs. However, ADH1, ADH3 and ADH4 predominate in rat skin, demonstrate different sensitivities to pyrazole, and are responsible for metabolizing glycol ethers. ALDH1 is the predominant isoform in rat liver and skin cytosolic fractions that is selectively inhibited by disulfiram and responds to the amt. of aldehyde formed by the ADH isoforms expressed in these tissues. Thus, the different affinity of ADH and ALDH for alcs. and glycol ethers of different carbon-chain length may reflect the relative isoform expression in rat liver and skin. Following multiple topical exposure, ethanol metab. increased the most following ethanol treatment, and 2-BE metab. increased the most following 2-BE treatment. Ethanol and 2-BE may induce specific ADH and ALDH isoforms that preferentially metabolize short-chain alcs. (i.e., ADH1, ALDH1) and longer chain alcs. (i.e., ADH3, ADH4, ALDH1), resp. Treatment with a general inducing agent such as dexamethasone enhanced ethanol and 2-BE metab. suggesting induction of multiple ADH isoforms. OS.CITING REF COUNT: 22 THERE ARE 22 CAPLUS RECORDS THAT CITE THIS RECORD (22 CITINGS)

18. Long, P. H., et al. (2000). "Dental pulp infarction in female rats following inhalation exposure to 2-butoxyethanol." Toxicol Pathol **28**(2): 246-252.

Female Fischer 344 (F344)/N rats (10 per exposure group) were exposed to 2-butoxyethanol (BE) vapors (0, 31, 62.5, 125, 250, or 500 ppm 6 h/d, 5 d/wk, for 13 wk) to characterize its prechronic toxicity. Dental lesions consisting of bilateral multifocal dental pulp thrombosis, pulp infarction, and odontoblast infarction were noted in the maxillary incisors of 3 of 4 rats from the 500-ppm group that were sacrificed when moribund during the first week of exposure. In addn., 1 rat from the 500-ppm group that was sacrificed on day 32 had similar unilateral incisor lesions but with addnl. findings consistent with a unilateral maxillary incisor fracture. In contrast, rats sacrificed

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after 13 wk of exposure lacked dental lesions. In conclusion, BE has the potential to cause pulp thrombosis and odontoblast infarction in female rats. The apparent variability in response to BE noted in moribund sacrificed vs terminally sacrificed rats was attributed to development of tolerance to BE-induced ***hemolysis*** and subsequent incisor regeneration. OS.CITING REF COUNT: 9 THERE ARE 9 CAPLUS RECORDS THAT CITE THIS RECORD (9 CITINGS)

19. Palkar, P. S., et al. (2007). "Priming dose of phenylhydrazine protects against hemolytic and lethal effects of 2-butoxyethanol." Toxicol Appl Pharmacol **225**(1): 102-112.

Protection against a high dose of a toxicant by prior exposure to another toxicant is called heteroprotection. Our objective was to establish a heteroprotection model in RBCs. Female Sprague Dawley rats treated with an LD90 dose of 2-butoxyethanol (BE, 1500 mg/kg in water, 5 mL/kg po) 14 days after priming with 0.9% NaCl suffered 90% mortality by 15 days, whereas all rats receiving the LD90 dose of BE 14 days after priming with phenylhydrazine (PHZ, 125 mg/kg in 0.9% NaCl, 3 mL/kg po) survived. Hematocrit decreased from normal 45% to 24% by day 3 after PHZ priming and improved thereafter. Increasing the time interval between the priming and LD90 dose to 21 days abolished the heteroprotection. RBCs obtained on days 7 and 14 after PHZ priming unlike those on day 21 were resilient to the hemotoxic metabolite of BE, butoxyacetic acid (BAA). Unaltered hepatic alc. and aldehyde dehydrogenase activities upon PHZ priming suggested that bioactivation of BE to BAA was unaffected. Lower renal (6 and 12 h) and hepatic (12 h) BAA levels and 3-fold higher excretion of BAA in PHZ-primed rat urine suggested a protective role of toxicokinetics. Higher erythropoietin, reticulocytes, and resiliency of PHZ-primed rat RBCs indicated that newly formed RBCs are resilient to hemolytic BAA. The antioxidant levels in the PHZ-primed rat RBCs did not indicate a protective role in heteroprotection. In conclusion, the resistance of PHZ-primed rats against BE-induced hemotoxicity and lethality is mediated by a combination of altered toxicokinetics, robust erythropoiesis, and resiliency of new RBCs. OS.CITING REF COUNT: 2 THERE ARE 2 CAPLUS RECORDS THAT CITE THIS RECORD (2 CITINGS)

20. Palkar, P. S. C. S. U. o. L. M. L. A. U. S. A. (2008). "Induction and proteomics of anti-hemolytic factors in rat red blood cells." (DA3313260). Dissertation.

21. Park, J., et al. (2002). "Mechanisms of 2-butoxyethanol carcinogenicity: studies on Syrian Hamster Embryo (SHE) cell transformation." Toxicological Sciences **68**(1): 43-50.

Previous studies showed that 2-butoxyethanol increased liver tumors in B6C3F1 mice following chronic exposure. While the mechanism of 2-butoxyethanol-induced ***liver*** ***carcinogenicity*** has not been defined, 2-butoxyethanol has been shown to induce ***hemolysis*** in rodents via 2-butoxyacetic acid, the major metabolite of 2-butoxyethanol. This toxic effect, coupled with the observation that continued treatment with 2-butoxyethanol results in hemosiderin deposition in the liver, has led to our hypothesis that ***liver*** ***carcinogenicity*** by 2-butoxyethanol is mediated via oxidative stress (iron catalyzed) and Kupffer cell activation. The present study used Syrian Hamster Embryo (SHE) cell transformation, a

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surrogate in vitro model for carcinogenesis in vivo, to examine whether 2-butoxyethanol, 2-butoxyacetic acid, or iron (ferrous sulfate) produced cell transformation. SHE cells were treated with either 2-butoxyethanol (0.5-20 mM), 2-butoxyacetic acid (0.5-20 mM), or ferrous sulfate (0.5-75 .mu.g/mL) for 7 days. 2-Butoxyethanol and 2-butoxyacetic acid did not induce cellular transformation. In contrast, treatment with ferrous sulfate (2.5 and 5.0 .mu.g/mL) increased morphol. transformation. Cotreatment of ferrous sulfate with the antioxidants .alpha.-tocopherol (vitamin E) or (-)-epigallocatechin-3-gallate (EGCG) prevented ferrous sulfate-induced transformation, suggesting the involvement of oxidative stress in SHE cell transformation. The level of oxidative DNA damage (OH8dG) increased following ferrous sulfate treatment in SHE cells; addnl., using single cell gel electrophoresis (comet assay), ferrous sulfate treatment produced an increase in DNA damage. Both DNA lesions were decreased by cotreatment of ferrous sulfate with antioxidants. These data support our proposal that iron, produced indirectly through ***hemolysis***, and not 2-butoxyethanol or its metabolite 2-butoxyacetic acid, is responsible for the obsd. carcinogenicity of 2-butoxyethanol. OS.CITING REF COUNT: 12 THERE ARE 12 CAPLUS RECORDS THAT CITE THIS RECORD (12 CITINGS)

22. Shabat, S., et al. (2004). "Osteonecrosis in a chemically induced rat model of human hemolytic disorders associated with thrombosis - a new model for avascular necrosis of bone." Calcified Tissue International **74**(3): 220-228.

Bone injury occurs in human hemolytic disorders assocd. with thrombosis, such as betathalassemia and sickle cell disease. Exposure of rats to 2-butoxyethanol (BE) has been assocd. with hemolytic anemia, disseminated thrombosis, and infarction in multiple organs including bone. This rat model apparently mimics acute ***hemolysis*** and thrombosis in humans. To elucidate the extent of bone injury, male and female Fischer F344 rats were given 4 daily doses of 250 mg BE/5 mL water/kg of body wt. Tail vertebrae were studied by histopathol. and magnetic resonance imaging (MRI). Thrombosis and infarction were seen in both sexes, but females were more severely affected. Lesions were characterized by extensive medullary fat necrosis, granulomatous inflammation, fibroplasia, growth plate degeneration, and new woven bone formation adjacent to necrotic bone trabeculae. MRI mean and std. deviation tissue-d. data for both sexes indicated a significant decrease following 4-days treatment and a significant increase following an addnl. 24 days without treatment. Thus, MRI was useful in revealing BE-induced bone injury, which was predominantly necrotic initially and subsequently regenerative with proliferation of connective tissue and bone following postischemia recovery. OS.CITING REF COUNT: 4 THERE ARE 4 CAPLUS RECORDS THAT CITE THIS RECORD (4 CITINGS)

23. Siesky, A. M., et al. (2002). "Hepatic effects of 2-butoxyethanol in rodents." Toxicological Sciences **70**(2): 252-260.

Chronic inhalation of 2-butoxyethanol resulted in an increase in liver hemangiosarcomas and hepatic carcinomas in male mouse liver. No increase in liver neoplasia was obsd. in similarly exposed male and female rats or female mice. The authors proposed that the prodn. of liver neoplasia in the male mouse is the result of oxidative damage secondary

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to the hemolytic deposition of iron in the liver. This occurs selectively in the male mouse and leads either directly or indirectly to liver neoplasia. To address this proposal, male B6C3F1 mice and male F344 rats were treated with 2-butoxyethanol (via daily gavage; 5 times per wk) at doses of 0, 225, 450, and 900 mg/kg/day (mice) and 0, 225, and 450 mg/kg/day (rats) resp. Following treatment for 7, 14, 28, and 90 days, DNA synthesis, oxidative damage, hematocrit, and iron deposition were measured in the livers. An increase in **hemolysis** (measured by a decrease in hematocrit and increase in relative spleen wt.) was obsd. in 2-butoxyethanol-treated rats and mice in a dose-dependent manner. An increase in the percentage of iron-stained **Kupffer cells** was obsd. following treatment with 450 and 900 mg/kg of 2-butoxyethanol in mice and 225 and 450 mg/kg of 2-butoxyethanol in rats. A biphasic increase in oxidative damage (8-hydroxydeoxyguanosine and malondialdehyde) was seen in mouse liver after 7 and 90 days of treatment with 2-butoxyethanol, whereas no increases were obsd. in treated rat liver. Vitamin E levels were reduced by 2-butoxyethanol treatment in both mice and rat liver; however, the basal level of vitamin E was approx. 2.5-fold higher in rat than in mouse liver. A similar biphasic induction of DNA synthesis was seen following 2-butoxyethanol treatment in the mouse. In the mouse liver, increased DNA synthesis was obsd. in hepatocytes at 90 days and in endothelial cells at 7 and 14 days at all doses. No change in DNA synthesis was seen in 2-butoxyethanol-treated rat liver. No apparent differences in apoptosis and mitosis in the liver were obsd. in mouse and rat liver between 2-butoxyethanol treatment groups and untreated controls. These results suggest that DNA synthesis, possibly from oxidative stress or Kupffer cell activation, occurs selectively in the mouse liver, primarily in endothelial cells (a target of 2-butoxyethanol neoplasia), following exposure to 2-butoxyethanol. OS.CITING REF COUNT: 18 THERE ARE 18 CAPLUS RECORDS THAT CITE THIS RECORD (18 CITINGS)

24. Starek, A., et al. (2007). "Pyrazole and methylpyrazole for the treatment of 2-butoxyethanol poisoning." Acta Pol Pharm **64**(1): 93-100.

2-Butoxyethanol (BE) is a one member of a family of ethylene glycol monoalkyl ethers that are used in a variety of industrial and household products. The clin. features of human and animal BE intoxications mainly include metabolic acidosis. CNS depression and coma, hemolytic anemia, hematuria, and renal injury. It is believed that metabolic activation of BE to butoxyacetic acid (BAA) is responsible for these pathol. changes. The treatment of BE poisoning have been based on an inhibition of the metabolic pathway enzymes which convert BE to toxic metabolites. Therefore, a comparison was made between antidotal properties of pyrazole (PY) and 4-methylpyrazole (MP) in rats s.c. intoxicated with BE. It was found that both antidotes effectively protected animals against appearance of hemolytic anemia signs induced by BE. MP appears to be more efficient than PY. These data confirm the beneficial role of alc. dehydrogenase (ADH) inhibitors in BE intoxication. OS.CITING REF COUNT: 3 THERE ARE 3 CAPLUS RECORDS THAT CITE THIS RECORD (3 CITINGS)

25. Starek, A. C. S. C. M. U. J. K. P. (2002). "2-Butoxyethanol. Documentation of proposed permissible values of occupational exposure levels." Podstawy i Metody Oceny Srodowiska Pracy **18**(4): 25-40.

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2-Butoxyethanol (BE) is a colorless liq. with an ether-like odor. BE is used as a solvent for nitrocellulose, both natural and synthetic resins, latex paints and varnishes, and insecticides, as a component of textile lubricants, cutting oils, and hydraulic brake fluids, and as a chem. intermediate. In Poland over 1000 workers are exposed to BE at level of 0-6.9 mg/m³. BE exerts an irritative effect on the eyes and upper respiratory tract and also causes central nervous system depression and intravascular ***hemolysis***. No carcinogenic, mutagenic, embryotoxic, fetotoxic, or teratogenic effects have been found in the relevant toxicol. studies. In subchronic inhalation expts., a hemolytic effect in rats was obsd. On the basis of this observation, NOAEL and LOAEL values of 125 mg/m³ and 385 mg/m³, resp., were proposed. A MAC value of 100 mg/m³ is recommended. An STEL value was established on the basis of irritation effect of BE in volunteers and proposed to be 200 mg/m³.

26. Travlos, G. S., et al. (2011). "Chronic progressive nephropathy in male F344 rats in 90-day toxicity studies: its occurrence and association with renal tubule tumors in subsequent 2-year bioassays." Toxicol Pathol **39**(2): 381-389.

The occurrence and severity of spontaneous chronic progressive nephropathy (CPN) in control male F344 rats as well as the frequency of treatment-related CPN exacerbation were histopathol. reevaluated. A series of 43 National Toxicol. Program (NTP) 90-day toxicity studies comparing the influence of NIH-07 or NTP-2000 diets was examd. Relationships between the histopathol. findings at 90 days and renal tubule proliferative lesions recorded in subsequent 2-yr bioassays for 24 chems. were statistically analyzed. CPN lesions were obsd. in 100% of the control male rats regardless of diet, but CPN was more severe in control rats fed NIH-07. Approx. one-third of the 90-day studies demonstrated a treatment-related exacerbation of CPN severity, which was independent of diet. For chems. that proceeded to 2-yr bioassays, all studies with a statistically significant increase in renal tubule tumors (RTT) at 2 years had treatment-related exacerbation of CPN in the 90-day and 2-yr studies. These findings indicate that CPN occurs ubiquitously in young male F344 rats and that treatment-related exacerbation of CPN in 90-day studies is a relatively common occurrence, having the potential to be predictive of an increased incidence of RTT in subsequent 2-yr bioassays. OS.CITING REF COUNT: 3 THERE ARE 3 CAPLUS RECORDS THAT CITE THIS RECORD (3 CITINGS)

27. Udden, M. M. C. S. D. o. M. B. C. o. M. H. T. X. U. S. A. (2000). "Rat erythrocyte morphological changes after gavage dosing with 2-butoxyethanol: a comparison with the in vitro effects of butoxyacetic acid on rat and human erythrocytes." Journal of Applied Toxicology **20**(5): 381-387.

Rats exposed to 2-butoxyethanol (2-BE) develop ***hemolysis*** proceeded by red blood cell swelling and shape changes. In this study, the effects on red blood cell morphol. of dosing rats with 2-BE by gavage were compared with the effects of incubation of rat erythrocytes in vitro with the principal metabolite of 2-BE, butoxyacetic acid (BAA). Morphol. was assessed by bright-field and phase microscopy of Wright's stain blood smears and glutaraldehyde-fixed cells suspended in plasma or buffer. In vivo exposure to 2-BE resulted in stomatocytosis and spherocytosis in blood smears and cup-shaped cells and spherocytosis in the fixed samples. In vitro incubation

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with BAA produced erythrocytes with cup shapes, spherocytosis, and red blood cell ghosts in fixed samples. The stomatocytes obsd. in the blood smears appear to be the morphol. equiv. of the cup-shaped cells obsd. in fixed samples. A variable degree of echinocytosis was obsd. in blood smears from animals exposed to 2-BE and in the in vitro expts. with BAA. Stomatocytes, cup-shaped cells, and spherocytes are the principal morphol. features of erythrocytes from rats exposed to 2-BE or in vitro exposure to BAA. In comparison, human red blood cells incubated with up to 2.0 mM BAA exhibited none of the morphol. changes obsd. in rat erythrocytes. 2-Butoxyethanol in vivo and BAA in vitro cause similar changes in rat red blood cell morphol., adding further evidence to support the primary role of BAA in the hemolytic effect of 2-BE exposure in the rat. OS.CITING REF COUNT: 22 THERE ARE 22 CAPLUS RECORDS THAT CITE THIS RECORD (22 CITINGS)

28. Udden, M. M. C. S. D. o. M. H. O. S. B. C. o. M. H. T. X. U. S. A. (2002). "In Vitro Sub-Hemolytic Effects of Butoxyacetic Acid on Human and Rat Erythrocytes." Toxicological Sciences **69**(1): 258-264.

When 2-butoxyethanol (2-BE) is administered to rats, ***hemolysis*** occurs as the active metabolite butoxyacetic acid (BAA) is formed. Human red blood cells appear to be relatively resistant to the hemolytic effects of BAA in vitro, whereas rat red blood cells undergo changes in deformability, cell swelling, and ***hemolysis***. In this study, exposure of human red blood cells to high concns. of BAA resulted in loss of deformability and a small increase in mean cellular vol., but no significant ***hemolysis***. These changes resembled the changes that occur in rat erythrocytes exposed to much lower concns. of BAA. Therefore, a comparison was made between the sub-hemolytic effects of BAA at high concns. (up to 10 mM) on human red cells with the sub-hemolytic effects of lower concns. of BAA (up to 0.1 mM) on rat erythrocytes. Under these conditions, human and rat erythrocyte deformability decreased, while mean cellular vol. (MCV) and osmotic fragility increased. Although there was a substantial shift in rat erythrocytes to lower densities, human erythrocyte d. was only slightly decreased. Human and rat erythrocyte sodium also increased. Rat erythrocytes demonstrated increased spherocytosis. In a survey of blood samples from adults and children, none demonstrated an increase in ***hemolysis*** (n = 97) or MCV (n = 65) after exposure to 10 mM BAA for 4 h. In these expts., in which ***hemolysis*** was not evident, human erythrocytes required exposure to a 100-fold greater concn. of BAA to develop changes in red cell deformability, osmotic fragility, and sodium content similar to those obsd. in rat erythrocytes. These concns. are not likely to occur under normal human use of 2-BE-contg. products. OS.CITING REF COUNT: 27 THERE ARE 27 CAPLUS RECORDS THAT CITE THIS RECORD (27 CITINGS)

29. Zeiger, E. C. S. E. T. P. N. I. o. E. H. S. R. T. P. N. C. U. S. A. (2000). "An interesting state of affairs in genetic toxicology." Environmental and Molecular Mutagenesis **35**(2): 82-85 CODEN: EMMUEG; ISSN: 0893-6692 PUBLISHER: Wiley-Liss.

The field of genetic toxicity testing was built and has been sustained on the hypothesis that mutagenicity portends carcinogenicity and nonmutagenicity portends a lower risk of carcinogenicity; the data was originally developed from Salmonella test. This

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hypothesis was weakened somewhat by studies from the U.S. National Toxicol. Program (NTP) database that showed a lower sensitivity to carcinogens. In the NTP, and other testing programs, there is often a selection bias against the strongly mutagenic and clastogenic chems. that are anticipated to be rodent carcinogens. There is no dispute that mutation is the initiating, and/or a promoting event in most cancers. OS.CITING REF COUNT: 7 THERE ARE 7 CAPLUS RECORDS THAT CITE THIS RECORD (7 CITINGS)

30. Zeiger, E. C. S. E. T. P. N. I. o. E. H. S. R. T. P. N. C. U. S. A. (2000). "An interesting state of affairs in genetic toxicology. [Erratum to document cited in CA132:344193]."
Environmental and Molecular Mutagenesis **36**(2): 161 CODEN: EMMUEG; ISSN: 0893-6692 PUBLISHER: Wiley-Liss.

PubMed/MEDLINE

Years of Coverage: approximately 1949 to the present, with some older material

PubMed was searched on Sept. 27, 2013 by Megan Goucher, Toxicology & Environmental Research and Consulting (TERC) of The Dow Chemical Company.

Terms used in the search:

Butoxyethanol, 111-76-2[rm], MOA, Hemolysis, Alkoxyacetic Acid, Hemolysis, Carcinogenicity, Genotoxicity, Kupffer cells

Limits applied:

Publication years: 2000 - Present

The search resulted in 23 hits.

1. Amir, G., et al. (2011). "2-Butoxyethanol model of haemolysis and disseminated thrombosis in female rats: a preliminary study of the vascular mechanism of osteoarthritis in the temporomandibular joint." Br J Oral Maxillofac Surg **49**(1): 21-25.

Female rats develop haemolytic anaemia and disseminated thrombosis and infarction in multiple organs, including bone, when exposed to 2-butoxyethanol (BE). There is growing evidence that vascular occlusion of the subchondral bone may play a part in some cases of osteoarthritis. The subchondral bone is the main weight bearer as well as the source of the blood supply to the mandibular articular cartilage. Vascular occlusion is thought to be linked to sclerosis of the subchondral bone associated with disintegration of the articular cartilage. The aim of this study was to find out whether this model of haemolysis and disseminated thrombosis supports the vascular hypothesis of osteoarthritis. Six female rats were given BE orally for 4 consecutive days and the two control rats were given tap water alone. The rats were killed 26 days after the final dose. The mandibular condyles showed histological and radiological features consistent with osteoarthritis in three of the four experimental rats and in neither of the control rats. These results may support the need to explore the vascular mechanism of osteoarthritis further.

2. Boatman, R., et al. (2004). "Review of studies concerning the tumorigenicity of 2-butoxyethanol in B6C3F1 mice and its relevance for human risk assessment." J Toxicol Environ Health B Crit Rev **7**(5): 385-398.

The U.S. National Toxicology Program (NTP) has completed 2-yr inhalation exposures in rats and mice with 2-butoxyethanol (BE). This review concerns the most significant findings from those studies and describes recent research into the mechanistic aspects of BE-mediated tumorigenesis in the mouse and the relevance of such effects to humans. Two tumor types were increased in B6C3F1 mice leading to the classification of "some evidence" of carcinogenicity: liver hemangiosarcomas in male mice and forestomach tumors in female mice (primarily benign papillomas). The results of research collected to date indicate that the tumorigenesis noted for BE was produced by

indirect mechanisms. In particular, the occurrence of liver hemangiosarcomas in male mice has been linked to oxidative damage subsequent to red blood cell hemolysis and iron deposition in this organ. Oral administration of BE in mice up to 600 mg/kg/d for up to 90 d produces a dose-related increase in iron (Perl's staining) in Kupffer cells and hepatocytes, increased DNA synthesis in endothelial cells, and enhanced oxidative damage. Further, iron alone, and not BE or BAA, is responsible for producing oxidative damage in cultured hepatocytes from rats or mice. Forestomach neoplasms in female mice were most likely a result of prolonged exposure-induced irritation with compensatory hyperplasia and subsequent tumor promotion. This mechanism is supported by studies indicating elevated levels of BE and BAA in the mouse forestomach tissues and stomach contents following multiple routes of exposure, forestomach epithelial cell cytotoxicity and cell proliferation following administration of BE and BAA, and the increased capacity of forestomach tissues from female mice to metabolize BE to the more irritating metabolite, BAA. The current article summarizes the results of a number of in vivo and in vitro studies designed to elucidate the underlying mechanisms of tumorigenesis by BE in the mouse and discusses the relevance of these for human risk.

3. Cavalcanti, B. C., et al. (2008). "Cytotoxicity and genotoxicity of ingenamine G isolated from the Brazilian marine sponge *Pachychalina alcaloidifera*." *Comp Biochem Physiol C Toxicol Pharmacol* **147**(4): 409-415.

Marine sponges belonging to the order Haplosclerida are one of the more prolific sources of new natural products possessing various biological activities. The present study examined the cytotoxic and genotoxic potential of ingenamine G, an alkaloid isolated from the Brazilian marine sponge *Pachychalina alcaloidifera*. Ingenamine G displayed a moderate cytotoxic activity against human proliferating lymphocytes evaluated by the MTT assay (IC₅₀ 15 microg/mL). The hemolytic assay showed that ingenamine G cytotoxic activity was not related to membrane disruption. The comet assay and chromosome aberration analysis were applied to determine the genotoxic and clastogenic potential of ingenamine G, respectively. Cultured human lymphocytes were treated with 5, 10, 15 and 20 microg/mL of ingenamine G during the G(1), G(1)/S, S (pulses of 1 and 6 h), and G(2) phases of the cell cycle. All tested concentrations were cytotoxic, reduced significantly the mitotic index, and were clastogenic in all phases of the cell cycle, especially in S phase. While an increase in DNA-strand breaks was observed starting with the concentration corresponding to the IC₅₀. The presence of genotoxicity and polyploidy during interphase and mitosis, respectively, suggests that ingenamine G at high concentrations is clastogenic and indirectly affects the construction of mitotic fuse.

4. Chen, D., et al. (2012). "Biocompatibility of magnetic Fe(3)O(4) nanoparticles and their cytotoxic effect on MCF-7 cells." *Int J Nanomedicine* **7**: 4973-4982.

BACKGROUND: The objective of this study was to evaluate the synthesis and biocompatibility of Fe(3)O(4) nanoparticles and investigate their therapeutic effects when combined with magnetic fluid hyperthermia on cultured MCF-7 cancer cells.
METHODS: Magnetic Fe(3)O(4) nanoparticles were prepared using a coprecipitation method. The appearance, structure, phase composition, functional groups, surface charge, magnetic susceptibility, and release in vitro were characterized by transmission

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electron microscopy, x-ray diffraction, scanning electron microscopy-energy dispersive x-ray spectroscopy, and a vibrating sample magnetometer. Blood toxicity, in vitro toxicity, and genotoxicity were investigated. Therapeutic effects were evaluated by MTT [3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide] and flow cytometry assays. RESULTS: Transmission electron microscopy revealed that the shapes of the Fe(3)O(4) nanoparticles were approximately spherical, with diameters of about 26.1 +/- 5.2 nm. Only the spinel phase was indicated in a comparison of the x-ray diffraction data with Joint Corporation of Powder Diffraction Standards (JCPDS) X-ray powder diffraction files. The O-to-Fe ratio of the Fe(3)O(4) was determined by scanning electron microscopy-energy dispersive x-ray spectroscopy elemental analysis, and approximated pure Fe(3)O(4). The vibrating sample magnetometer hysteresis loop suggested that the Fe(3)O(4) nanoparticles were superparamagnetic at room temperature. MTT experiments showed that the toxicity of the material in mouse fibroblast (L-929) cell lines was between Grade 0 to Grade 1, and that the material lacked hemolysis activity. The acute toxicity (LD(50)) was 8.39 g/kg. Micronucleus testing showed no genotoxic effects. Pathomorphology and blood biochemistry testing demonstrated that the Fe(3)O(4) nanoparticles had no effect on the main organs and blood biochemistry in a rabbit model. MTT and flow cytometry assays revealed that Fe(3)O(4) nano magnetofluid thermotherapy inhibited MCF-7 cell proliferation, and its inhibitory effect was dose-dependent according to the Fe(3)O(4) nano magnetofluid concentration. CONCLUSION: The Fe(3)O(4) nanoparticles prepared in this study have good biocompatibility and are suitable for further application in tumor hyperthermia.

5. Corthals, S. M., et al. (2006). "Mechanisms of 2-butoxyethanol-induced hemangiosarcomas." Toxicol Sci **92**(2): 378-386.

Chronic exposure to 2-butoxyethanol increased liver hemangiosarcomas in male mice. The mechanism for the selective induction of hemangiosarcomas by 2-butoxyethanol is unknown but has been suggested to occur through non-DNA-reactive mechanisms. The occurrence of liver hemangiosarcomas in male mice has been linked to oxidative damage subsequent to RBC hemolysis and iron deposition and activation of macrophages (Kupffer cells) in the liver, events that exhibit a threshold in both animals and humans. 2-Butoxyethanol is metabolized to 2-butoxyacetaldehyde and 2-butoxyacetic acid, and although the aldehyde metabolite is short lived, the potential exists for this metabolite to cause DNA damage. The present study examined whether 2-butoxyethanol and its metabolites, 2-butoxyacetaldehyde and 2-butoxyacetic acid, damaged mouse endothelial cell DNA using the comet assay. No increase in DNA damage was observed following 2-butoxyethanol (1-10mM), 2-butoxyacetaldehyde (0.1-1.0mM), or 2-butoxyacetic acid (1-10mM) in endothelial cells after 2, 4, or 24 h of exposure. Additional studies examined the involvement of hemolysis and macrophage activation in 2-butoxyethanol carcinogenesis. DNA damage was produced by hemolyzed RBCs (10 x 10⁶, 4 h), ferrous sulfate (0.1-1.0 microM; 2-24 h), and hydrogen peroxide (50-100 microM; 1-4 h) in endothelial cells. Hemolyzed RBCs also activated macrophages, as evidenced by increased tumor necrosis factor (TNF) alpha, while neither 2-butoxyethanol nor butoxyacetic acid increased TNF-alpha from macrophages. The effect of activated macrophages on endothelial cell DNA damage and DNA synthesis was also studied. Coculture of endothelial cells with activated macrophages increased endothelial cell DNA damage after 4 or 24 h and increased endothelial cell DNA

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synthesis after 24 h. These data demonstrate that 2-butoxyethanol and related metabolites do not directly cause DNA damage. Supportive evidence also demonstrated that damaged RBCs, iron, and/or products from macrophage activation (possibly reactive oxygen species) produce DNA damage in endothelial cells and that activated macrophages stimulate endothelial cell proliferation. These events coupled together provide the events necessary for the induction of hemangiosarcomas by 2-butoxyethanol.

6. Gross, A., et al. (2009). "Human aldehyde dehydrogenase-catalyzed oxidation of ethylene glycol ether aldehydes." *Chem Biol Interact* **178**(1-3): 56-63.

Ethylene glycol ethers (EGEs) are primary alcohols commonly used as solvents in numerous household and industrial products. Exposure to EGEs has been correlated with delayed encephalopathy, metabolic acidosis, sub-fertility and spermatotoxicity in humans. In addition, they also cause teratogenesis, carcinogenesis, hemolysis, etc., in various animal models. Metabolism EGEs parallels ethanol metabolism, i.e., EGEs are first converted to 2-alkoxy acetaldehydes (EGE aldehydes) by alcohol dehydrogenases, and then to alkoxyacetic acids by aldehyde dehydrogenases (ALDHs). The acid metabolite of EGEs is considered responsible for toxicities associated with EGEs. The role of human ALDHs in EGE metabolism is not clear; accordingly, we have investigated the ability of five different human ALDHs (ALDH1A1, ALDH2, ALDH3A1, ALDH5A1 and ALDH9A1) to catalyze the oxidation of various EGE aldehydes. The EGE aldehydes used in this study were synthesized via Swern oxidation. All of the human ALDHs were purified from human cDNA clones over-expressing these enzymes in *E. coli*. The ALDHs tested, so far, differentially catalyze the oxidation of EGE aldehydes to their corresponding acids ($K(m)$ values range from approximately 10 μ M to approximately 20.0mM). As judged by $V(max)/K(m)$ ratios, short-chain alkyl-group containing EGE aldehydes are oxidized to their acids more efficiently by ALDH2, whereas aryl- and long-chain alkyl-group containing EGE aldehydes are oxidized to their acid more efficiently by ALDH3A1. Given the product of ALDH-catalyzed reaction is toxic, this process should be considered as a bio-activation (toxification) process.

7. Kamendulis, L. M., et al. (2010). "Kupffer cells participate in 2-butoxyethanol-induced liver hemangiosarcomas." *Toxicology* **270**(2-3): 131-136.

2-Butoxyethanol increases hemangiosarcomas selectively in male mouse liver after chronic inhalation through mechanisms that have not fully been elucidated. Hemolysis, a primary toxic effect associated with 2-butoxyethanol exposure in rodents, increased hemosiderin (iron) deposition in Kupffer cells in the liver. These findings, along with the induction of hepatic neoplastic lesions, led to our hypothesis that the induction hemangiosarcomas by 2-butoxyethanol is due to the activation of Kupffer cells, subsequent to hemolysis, that results in the induction of DNA synthesis in target cells (endothelial cells); allowing for the selective proliferation of preneoplastic target cells and/or the promotion of new initiated cells. The present studies were conducted to determine whether Kupffer cells contributed to 2-butoxyethanol-induced endothelial DNA synthesis in the liver, thereby determining whether a linkage exists between these events. Male B6C3F1 mice were treated with 450 and 900 mg/kg 2-butoxyethanol (via daily gavage; 5x/week) for 7 days in the presence or absence of Kupffer cell depletion (via clodronate-encapsulated liposomes). 2-Butoxyethanol (450 and 900 mg/kg/day)

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increased the number of F4/80 stained cells (Kupffer cells) compared to controls (approximately 1.3- and approximately 1.6-fold over control, respectively). Clodronate liposome treatment reduced the number of Kupffer cells by >90%, as assessed by F4/80 immunohistochemistry. Increased hemolysis, measured by increases in relative spleen weights and decreased hematocrit was confirmed in 2-butoxyethanol treated mice. The percentage of iron-stained endothelial cells increased by approximately 11-fold over control, and endothelial cell DNA synthesis increased approximately 1.7-fold over control in 2-butoxyethanol exposed mice. Importantly, Kupffer cell depletion reduced 2-butoxyethanol-induced iron staining and hepatic endothelial cell DNA synthesis. These studies provide evidence supporting the hypothesis that the Kupffer cell modulates 2-butoxyethanol-induced endothelial cell DNA synthesis, and therefore may contribute to hemangiosarcoma induction by 2-butoxyethanol.

8. Klajnert, B., et al. (2006). "Cytotoxicity, haematotoxicity and genotoxicity of high molecular mass arborescent polyoxyethylene polymers with polyglycidol-block-containing shells." Cell Biol Int **30**(3): 248-252.

We have examined the impact on biological systems of some newly synthesised polyoxyethylene polymers using in vitro assays. Toxicity was tested by the 3-[4,5-dimethylthiazole-2-yl]-2,5 diphenyltetrazolium bromide (MTT) assay, haemolysis was assessed, and an ethidium bromide (EB) assay was used to study interactions between the polymers and DNA. All the assay data showed that the polymers are biocompatible. No differences were found between generations (i.e. macromolecule sizes). The results encourage continuing studies on the clinical use of these molecules as drug carriers.

9. Klaunig, J. E. and L. M. Kamendulis (2005). "Mode of action of butoxyethanol-induced mouse liver hemangiosarcomas and hepatocellular carcinomas." Toxicol Lett **156**(1): 107-115.

Chronic exposure to 2-butoxyethanol resulted in an increase in liver hemangiosarcomas and hepatic carcinomas in male mouse liver. No increase in liver neoplasia was observed in similarly exposed male and female rats or female mice. We have proposed that the production of liver neoplasia in the male mouse is the result of oxidative damage secondary to the hemolytic deposition of iron in the liver. Our working hypothesis is that the mode of action of butoxyethanol-induced mouse liver hemangiosarcomas and hepatic neoplasia involves the metabolism of 2-butoxyethanol to butoxyacetic acid which results in the induction of RBC hemolysis. This hemolytic response is translated into the accumulation of iron in both liver hepatocytes and Kupffer cells. The Kupffer cell response to this insult is two-fold: (1) the production of oxidative species-through both Kupffer cell activation and through the Fenton reaction involving iron and (2) the production of cytokines (for example TNF alpha). The induction of reactive oxygen species can, if not scavenged, produce oxidative DNA damage (the formation of OH8dG), as well as increase cell growth through modulation of gene expression. While the reactive oxygen species generation would occur in the both rats and mice, the ability of the rat to detoxify the reactive oxygen species would preclude the remaining steps from occurring. In contrast, in the mouse, the reactive oxygen species would override antioxidant defense mechanisms and allow the proposed mode of action to move forward. Our results to date in male B6C3F1 mice and male F344 rats treated with 2-

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butoxyethanol (via daily gavage; five times per week) at doses of 0, 225, 450, and 900 mg/kg/day (mice) and 0, 225, 450 mg/kg/day (rats), respectively, showed: an increase in hemolysis in 2-butoxyethanol treated rats and mice in a dose-dependent manner, in addition, an increase in the percent of iron stained Kupffer cells in the liver was observed following treatment with 450 and 900 mg/kg of 2-butoxyethanol in mice and 225 and 450 mg/kg of 2-butoxyethanol in rat. With the iron deposition, a biphasic increase in oxidative damage (OH8dG and malondialdehyde) was seen in mouse liver after treatment with 2-butoxyethanol. In contrast, no increase in oxidative damage was observed in the rat liver at any of the doses examined. Concomitant with the increase in oxidative damage, Vitamin E levels were similarly reduced by 2-butoxyethanol in both mice and rat liver. However, the basal level of Vitamin E in rat liver was 2.5-fold greater than in mouse liver. A biphasic induction of DNA synthesis was seen following 2-butoxyethanol in the mouse. In mouse liver, increased DNA synthesis was observed in hepatocytes at 90 days and in endothelial cells at 7 and 14 days at all doses. No change in DNA synthesis was seen in 2-butoxyethanol treated rat liver. No apparent differences in apoptosis and mitosis in the liver were observed in mouse and rat liver between 2-butoxyethanol treatment groups and untreated controls. These results suggest that the induction of DNA synthesis, possibly from oxidative stress and/or Kupffer cell activation, occurs selectively in the mouse liver, in endothelial cells and in hepatocytes following exposure to 2-butoxyethanol, and support the hypothesis proposed above.

10. Koshkaryev, A., et al. (2003). "2-Butoxyethanol enhances the adherence of red blood cells." Arch Toxicol **77**(8): 465-469.

We recently presented a unique, chemically-induced rat model of hemolytic anemia and disseminated thrombosis. In this 2-butoxyethanol (BE)-induced model the organs developing infarction are comparable to those seen in human diseases, characterized by hemolysis and thrombosis (e.g., thalassemia, sickle-cell disease, paroxysmal nocturnal hemoglobinuria, disseminated intravascular coagulation, thrombotic thrombocytopenic purpura, and hemolytic uremic syndrome). Red blood cells (RBCs) have special flow properties, namely, self-aggregability, deformability, and potential adherence to endothelial cells (ECs) of the blood vessel wall, which are essential for adequate blood flow and tissue perfusion; their alteration facilitates circulatory disorders. To examine the possible contribution of alterations in RBC flow properties to the observed thrombosis in the present investigation we determined the BE-induced changes in adherence, aggregability, and deformability of RBCs from male and female Fischer F344 rats exposed to two, three, or four daily doses of BE at 250 mg BE/kg body weight. Control animals were treated with the vehicle alone. Blood was taken on days 2, 3, 4, and 29. The administration of BE did not affect the RBCs aggregability but markedly enhanced their adherence to extracellular matrix; such enhancement was correlated with adherence to cultured ECs. RBC/EC interaction has been shown to be a potent catalyst of vascular occlusion in hemolytic hemoglobinopathies; thus the enhanced RBC adherence to EC is a likely mechanism by which thrombosis and organ infarct are induced in BE-treated rats.

11. Lewis, D. N., et al. (2005). "2-Butoxyethanol female-rat model of hemolysis and disseminated thrombosis: X-ray characterization of osteonecrosis and growth-plate suppression." Toxicol Pathol **33**(2): 272-282.

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We recently proposed a chemically induced rat model for human hemolytic disorders associated with thrombosis. The objective of the present investigation was to apply a noninvasive, high-magnification X-ray analysis, the Faxitron radiography system, to characterize the protracted bone damage associated with this 2-butoxyethanol model and to validate it by histopathology. Groups of female Fischer 344 rats were given 0, 250, or 300 mg of 2-butoxyethanol/kg body weight daily for 4 consecutive days. Groups were then sacrificed 2 hours or 26 days after the final treatment. The treated animals displayed a darkened purple-red discoloration on the distal tail. Histopathological evaluation, including phosphotungstic acid-hematoxylin staining of animals sacrificed 2 hours after the final treatment, revealed disseminated thrombosis and infarction in multiple organs, including bones. The Faxitron MX-20 specimen radiography system was used to image selected bones of rats sacrificed 26 days posttreatment. Premature thinning of the growth plate occurred in the calcaneus, lumbar and coccygeal vertebrae, femur, and ilium of the treated animals. Areas of decreased radiographic densities were seen in the diaphysis of the femur of all treated animals. The bones were then examined histologically and showed a range of changes, including loss or damage to growth plates and necrosis of cortical bone. No thrombi were seen in the animals sacrificed at 30 days, but bone and growth plate changes consistent with prior ischemia were noted. The Faxitron proved to be an excellent noninvasive tool that can be used in future studies with this animal model to examine treatment modalities for the chronic effects of human thrombotic disorders.

12. Narayanan, S., et al. (2001). "Lysis of whole blood in vitro causes DNA strand breaks in human lymphocytes." Mutagenesis **16**(6): 455-459.

DNA damage in lymphocytes, as measured by alkaline single cell gel electrophoresis (pH 12.7), is greatly increased by the concurrent lysis of whole blood in both freshly isolated samples and in PHA-stimulated cultures over a period of 7 days. Further, there is a marked progressive increase in DNA damage with time in PHA-stimulated lymphocytes cultured in whole blood even when the lymphocytes are separated before analysis; no such increase is seen in lymphocytes cultured alone. This indicates that there are components in whole blood that can cause DNA damage in lymphocytes, with granulocytes and lysis of red blood cells likely candidates. The DNA damage is greatly reduced in granulocyte-depleted whole blood cultures, but even in these significant increases are seen at later sampling times. Consequently, careful sample preparation is of paramount importance if the Comet assay is to be successfully used to assess DNA damage in human peripheral blood lymphocytes. Further, the progressive increase in DNA damage in whole blood cultures may influence other methods using lymphocytes for population biomonitoring and may be significant for in vitro genotoxicity testing.

13. Nyska, A., et al. (2003). "Ocular expression of vascular cell adhesion molecule (VCAM-1) in 2-butoxyethanol-induced hemolysis and thrombosis in female rats." Exp Toxicol Pathol **55**(4): 231-236.

We demonstrated previously that exposure of rats to 2-butoxyethanol (BE) was associated with morphological changes in red blood cells, hemolytic anemia, and disseminated thrombosis and infarction in different organs including the eyes. In order

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to elucidate the mechanism of thrombosis formation, we examined in this study the histology and immunohistochemical expression of vascular cell adhesion molecule-1 (VCAM-1), endothelial intercellular adhesion molecule-1 (ICAM-1), and P-selectin in the eyes of the female F344 rat exposed to 2, 3, or 4 daily doses of BE/250 mg/kg body weight. In this BE hemolysis and thrombosis model, positive VCAM-1 expression occurred only in eyes of rats exposed to 3 and 4 doses and was localized in the iris (epithelium lining the posterior surface, anterior mesenchymal epithelium), ciliary processes (lining epithelium, stromal cells), and retina (hypertrophic retinal pigment epithelium). Only weak immunolabeling was seen in eyes exposed to 2 doses. The appearance of VCAM-1 immunostaining correlated with the development of thrombosis located in the same structures. No change in ICAM-1 or P-selectin expression was seen. This immunolabeling distribution suggests that VCAM-1 functions in the pathogenesis of BE-related thrombosis by promoting adhesion of erythrocytes to the endothelium.

14. Palkar, P. S., et al. (2008). "The rat red blood cell proteome is altered by priming with 2-butoxyethanol." Toxicol Appl Pharmacol **230**(3): 338-345.

Administration of a low priming dose of 2-butoxyethanol (BE, 500 mg/kg, p.o.) 7 days prior to a larger LD(90) dose (1500 mg BE/kg, p.o.) offers protection against the lethal dose-induced hemolysis and death in female Sprague Dawley rats because of prompt and efficient replacement of red blood cells (RBCs) with new resilient RBCs. The objective of the present work was to analyze the altered proteome of RBCs upon priming with BE in order to identify the potential anti-hemolytic survival proteins induced in the primed rat RBCs (P-RBCs) as opposed to vehicle-treated RBCs (V-RBCs). The RBCs from the two groups were fractionated into membrane and cytosolic fractions. The cytosolic fractions were further fractionated for proteomic analysis into 3 fractions. The fractions were labeled with Cy3 and Cy5 fluorescent dyes and subjected to 2-dimensional differential gel electrophoresis (DIGE) to analyze the protein profiles. Seven membrane and 8 cytosolic proteins were found to be significantly increased (> or =2.5 fold) in P-RBCs as compared to V-RBCs. The identified proteins can be classified into antioxidant, membrane skeleton, protein turnover, lipid raft, and energy metabolism components. Increased levels of the proteins from antioxidant and membrane skeleton groups were confirmed by Western blot analysis. The study provides the first report on protein profiling of rat RBCs as well as on alteration of the proteome upon exposure to a priming dose of hemotoxicant. Further studies are needed to prove the protective role of the identified proteins and will initiate the field of survival/protective/anti-hemolytic proteins in RBCs.

15. Park, J., et al. (2002). "Effects of 2-butoxyethanol on hepatic oxidative damage." Toxicol Lett **126**(1): 19-29.

2-Butoxyethanol has been reported to induce an increase in liver tumors in male B6C3F1 mice following chronic inhalation while rats, similarly treated, showed no increase in liver tumors. The mechanism for the selective induction of cancer in mouse liver is unknown, however, 2-butoxyethanol has been shown to induce hemolysis in mice, resulting in an accumulation of hemosiderin (iron) in the liver. Previous studies by our group and others have shown that mouse liver compared to other rodent species has a lower antioxidant capacity and appears to be more susceptible to chemically-induced

oxidative damage. Since iron is known to produce hydroxyl radicals (through the Fenton reaction), we have proposed that the 2-butoxyethanol-induced iron overload (through hemolysis) may contribute to the induction of liver neoplasia in the mouse. In the present studies, 2-butoxyethanol induced oxidative stress in the liver of mice following 7-day treatment by gavage. These studies also examined whether 2-butoxyethanol, 2-butoxy acetic acid (a major metabolite of 2-butoxyethanol) or iron (FeSO₄) produced oxidative stress in mouse and rat hepatocytes. Oxidative stress was examined by measuring oxidative DNA damage (OH8dG), lipid peroxidation (MDA formation) and cellular vitamin E concentrations. Neither 2-butoxyethanol or 2-butoxyacetic acid induced changes in the oxidative stress parameters examined in either rat or mouse hepatocytes. In contrast, FeSO₄ produced a dose-related increase in OH8dG and MDA and a decrease in vitamin E levels following 24 h treatment. Mouse hepatocytes were more sensitive than rat hepatocytes to the oxidative damage induced by the FeSO₄. FeSO₄-induced oxidative stress was not increased by co-treatment of FeSO₄ with either 2-butoxyethanol or 2-butoxy acetic acid. These results support the proposal that the induction of hepatic oxidative stress by 2-butoxyethanol in vivo occurs secondary to induction of hemolysis and iron deposition in the liver rather than as a direct action of 2-butoxyethanol or its main metabolite, 2-butoxy acetic acid.

16. Park, J., et al. (2002). "Mechanisms of 2-butoxyethanol carcinogenicity: studies on Syrian Hamster Embryo (SHE) cell transformation." *Toxicol Sci* **68**(1): 43-50.

Previous studies showed that 2-butoxyethanol increased liver tumors in B6C3F1 mice following chronic exposure. While the mechanism of 2-butoxyethanol-induced liver carcinogenicity has not been defined, 2-butoxyethanol has been shown to induce hemolysis in rodents via 2-butoxyacetic acid, the major metabolite of 2-butoxyethanol. This toxic effect, coupled with the observation that continued treatment with 2-butoxyethanol results in hemosiderin deposition in the liver, has led to our hypothesis that liver carcinogenicity by 2-butoxyethanol is mediated via oxidative stress (iron catalyzed) and Kupffer cell activation. The present study used Syrian Hamster Embryo (SHE) cell transformation, a surrogate in vitro model for carcinogenesis in vivo, to examine whether 2-butoxyethanol, 2-butoxyacetic acid, or iron (ferrous sulfate) produced cell transformation. SHE cells were treated with either 2-butoxyethanol (0.5-20 mM), 2-butoxyacetic acid (0.5-20 mM), or ferrous sulfate (0.5-75 microg/ml) for 7 days. 2-Butoxyethanol and 2-butoxyacetic acid did not induce cellular transformation. In contrast, treatment with ferrous sulfate (2.5 and 5.0 microg/ml) increased morphological transformation. Cotreatment of ferrous sulfate with the antioxidants alpha-tocopherol (vitamin E) or (-)-epigallocatechin-3-gallate (EGCG) prevented ferrous sulfate-induced transformation, suggesting the involvement of oxidative stress in SHE cell transformation. The level of oxidative DNA damage (OH8dG) increased following ferrous sulfate treatment in SHE cells; additionally, using single cell gel electrophoresis (comet assay), ferrous sulfate treatment produced an increase in DNA damage. Both DNA lesions were decreased by cotreatment of ferrous sulfate with antioxidants. These data support our proposal that iron, produced indirectly through hemolysis, and not 2-butoxyethanol or its metabolite 2-butoxyacetic acid, is responsible for the observed carcinogenicity of 2-butoxyethanol.

17. Poet, T. S., et al. (2003). "Mode of action and pharmacokinetic studies of 2-butoxyethanol in the mouse with an emphasis on forestomach dosimetry." Toxicol Sci **71**(2): 176-189.

Chronic inhalation studies with 2-butoxyethanol (BE) conducted by the National Toxicology Program identified the forestomach and liver of B6C3F1 mice as target organs for tumorigenicity (NTP, 2000). Previous studies have shown that the liver tumors likely resulted from chronic hemolysis-induced oxidative stress. For the forestomach lesions seen in mice, chronic contact irritation (cytotoxicity) and regenerative hyperplasia are hypothesized to result in forestomach tumor development. To test this hypothesis, several experiments were conducted to address the sensitivity of the mouse forestomach to BE administered by various routes. Oral administration of undiluted BE was shown to cause irritation and a compensatory proliferative response in the mouse forestomach, confirming that direct contact between the forestomach and BE, which can occur via grooming of BE condensed on the fur during inhalation exposures, can cause irritation. However, only small amounts of BE (<10 mg/kg) were detected on the fur of mice at the end of 6-h, whole-body or nose-only inhalation exposures to the highest concentration used in the NTP chronic inhalation studies (250 ppm). Furthermore, no significant differences were detected in the end-exposure blood concentrations of BE and butoxyacetic acid (BAA) between these types of exposures. In addition, parenteral administration of BE (ip and sc injection) also resulted in forestomach lesions, indicating that there may be sources other than grooming for BE- or BAA-induced forestomach irritation. In the pharmacokinetic study, BE and, to a lesser extent, BAA was eliminated more slowly from the forestomach tissue of mice than from blood or other tissues, following either oral gavage or ip injection. The forestomach was the only tissue with detectable levels of BE at 24 h. BE and BAA were both excreted in the saliva and were present in stomach contents for a prolonged period of time following these routes of exposure, which may further contribute to forestomach tissue dosimetry. Thus, there appear to be multiple mechanisms behind the increased levels of BE and BAA in the forestomach tissue of mice, which together can contribute to a prolonged contact irritation, compensatory hyperplasia, and tumorigenicity in mice. The relevance of these effects in humans, who lack a forestomach, is questionable.

18. Starek, A. (2008). "Studies on the nature of hemolytic effect induced by ethylene glycol alkyl ethers." Acta Pol Pharm **65**(4): 421-425.

The nature of hemolytic effect induced by ethylene glycol alkyl ethers was analyzed taking into account G-6-PDH activity, ATP, pyruvate and thiols levels in peripheral blood of rats treated with single doses of 2-ethoxyethanol and 2-butoxyethanol. In addition, the susceptibility to autoxidation of rat erythrocyte lipids was evaluated. A decrease of ATP level in a dose-dependent manner and an increase in protein- and nonprotein-bound sulfhydryl groups were observed. These results indicate that an acute hemolytic effect of ethylene glycol alkyl ethers is not associated with alterations in G-6-PDH activity and the susceptibility of erythrocyte lipids to autoxidation. Increases in protein- and nonprotein-bound sulfhydryl groups seem to indicate the selective hemolysis of the aged erythrocytes. The increase in pyruvate and thiol levels may protect erythrocytes against the appearance of oxidative stress.

19. Starek, A., et al. (2004). "Comparison of the hemolytic activity of isopropoxyethanol and phenoxyethanol." Int J Occup Med Environ Health **17**(3): 339-346.

OBJECTIVES: Administration of ethylene glycol monoalkyl ethers to rodents causes acute hemolytic anemia. Metabolic activation of these chemicals to alkoxyacetic acids is required to develop hemolytic effect. Current study was undertaken to compare the hemolytic activity of isopropoxyethanol (IPE) and phenoxyethanol (PhE) in male rats. The main goal of this study was to evaluate the role of alkyl and aryl group in hemolytic activity of ethylene glycol ethers. MATERIALS AND METHODS: Rats were treated subcutaneously with single doses of 0, 0.625, 1.25 and 2.5 mmol IPE/kg body weight or 0, 2.5, 5.0 and 10.0 mmol PhE/kg. At 0, 6, 24, 48, 144, 216, and 600 h after dosing, blood samples were collected from end tail of rats and various blood indices were measured. RESULTS: Administration of both chemicals resulted in a time- and dose-dependent swelling of erythrocytes as evidenced by an early increase in packed cell volumes and mean cell volume. Subsequently, red blood cells, total hemoglobin concentration, and packed cell volumes decreased when hemolysis progressed. Furthermore, an increase in plasma hemoglobin concentration and reticulocyte counts was observed. The onset of hemolysis induced by IPE was faster than that after PhE administration. The hemolytic activity of IPE was about tenfold higher in comparison with PhE. CONCLUSIONS: It is likely that the lower hemolytic activity of PhE is associated with inhibitory effect of aryl group on hemolytic action of this compound. Phenyl group, in contrast with alkyl moiety, represents electron acceptor system which exerts resonance and inductive effects and leads to changes in acid strength, also in case of phenoxyacetic acid, a metabolite of PhE.

20. Starek, A. and P. Nowak (2006). "The alkoxyacetic acids as calcium and magnesium chelating agents in vitro." Acta Pol Pharm **63**(2): 89-94.

Alkoxyacetic acids (AAAs) are known urinary metabolites of the corresponding ethylene glycol monoalkyl ethers with a wide range of industrial and domestic applications. Hemolysis is the principal toxic effect of AAAs in humans and animals. The mechanism of red-cell damage is not known. It is suggested that some disturbances in ion balance, mainly related to calcium are one of the reasons of hemolysis. No comparative studies in the available literature on the chelating properties of numerous AAAs in respect to calcium were found. Therefore, a comparison was made between chelating effects of five AAAs on calcium and magnesium in vitro. It was demonstrated that calcium was bound at lower AAAs concentrations than magnesium. The chelating effect of AAAs expressed by EC50 values was positively correlated with both pKa values and Log P values of the examined acids. The obtained data indicate that the acidity and hydrophilic properties are responsible for the chelating effect of AAAs on calcium and magnesium in vitro. These data do not provide an explanation for differences in the hemolytic activity of the examined compounds.

21. Starek, A., et al. (2008). "Comparison of the in vitro hemolytic effects produced by alkoxyacetic acids on human and rat erythrocytes." Int J Occup Med Environ Health **21**(2): 147-155.

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OBJECTIVES: The alkoxyacetic acids (AAAs) are urinary metabolites of alkoxyethanol solvents. It is well documented that these chemicals can cause acute hemolytic anemia in humans and laboratory animals. There are scarce data on the relative hemolytic activity of these acids. Likewise, information is lacking on the relationship between their hemolytic activity and physicochemical properties. The aim of this study was to compare the hemolytic activity of five AAAs in red blood cells (RBCs) derived from donors' blood and male Wistar rats. Moreover, the possible relationship between lipophilic and hemolytic activity of AAAs was also investigated. **MATERIALS AND METHODS:** The RBCs washed in TRIS buffer, pH 7.4, were adjusted to a packed cell volume (PCV) of about 20% and incubated in a water bath at 37 degrees C for 0-3 h in the presence of different concentrations of AAAs. The hemolytic effects, in terms of the changes in RBCs, PCV, mean corpuscular volume (MCV) and free hemoglobin (HGBfree) in incubation medium, were evaluated. Based on the dose-response relationship for RBCs, PCV and MCV, the effective concentration values (EC50) and their 95% confidence intervals (95% CI) were calculated. The octanol-water partition coefficient (log P) and distribution coefficient (log D) of AAAs were computed using PALLAS software. The correlation between log P and log D values for AAAs at pH 7.4 and their EC50 was analyzed. **RESULTS:** Human RBCs were 1.9-3.1 times more resistant to the hemolytic activity of AAAs than rat erythrocytes. Also, the hemolytic activity of individual AAAs did not differ considerably; the maximum differences ranging from 2.0 to 3.3. The EC50 values of AAAs highly correlated with their log P and log D values. **CONCLUSIONS:** The relatively small differences between the hemolytic effects of AAAs on rat and human erythrocytes may be associated with the strong acidity and relatively similar lipophilicity of these chemicals.

22. Starek, A., et al. (2008). "Hematological effects of four ethylene glycol monoalkyl ethers in short-term repeated exposure in rats." *Arch Toxicol* **82**(2): 125-136.

This study was carried out to compare the hematological effects of 2-methoxyethanol (ME), 2-ethoxyethanol (EE), 2-isopropoxyethanol (IPE), and 2-butoxyethanol (BE) in short-term studies in rats. Male rats were subcutaneously treated with ME or EE at a dosage of 0, 1.25, 2.5 and 5.0 mM/kg in saline, 5 days per week, for 4 weeks. Other rats were exposed to IPE or BE at doses of 0, 0.25, 0.5, 0.75 and 1.25 mM/kg in the same manner. Administration of each chemical, except of ME, resulted in a time- and dose-dependent swelling of erythrocytes as evidenced by an increase in mean corpuscular volume (MCV). Subsequently, red blood cells (RBC), packed cell volumes (PCV), hemoglobin concentration (HGB), and mean cell hemoglobin concentration (MCHC) decreased. Furthermore, an increase in mean cell hemoglobin (MCH) and reticulocyte counts was observed. The onset of hemolysis induced by EE, IPE or BE was faster than after ME administration. While in rats exposed to ME hematological changes were strongly pronounced and progressively increased with exposure time beginning from the day 11, those in animals treated with EE were rather persisted at low constant level for all exposure period. In contrast, the rats exposed to IPE and BE demonstrated the dramatic hematological changes more pronounced in case of BE than IPE at the beginning of exposure (on day 4). Despite of exposure duration, these changes were regressed, although the decrease in RBC and MCHC and the increase in MCV and MCH in rats treated with highest doses of both compound (0.5, 0.75, and 1.25 mM/kg) were

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more persistent, probably due to selective hemolysis of the aged erythrocytes. In addition, significant leukopenia due to reduction of lymphocytes in rats exposed to ME was observed. In summary, this study demonstrated no tolerance to ME- and EE-induced intravascular hemolysis developed under these experimental conditions. On the contrary, tolerance to IPE- and BE-induced hemolysis in rats exposed to these compounds was prompted.

23. Wagner, S. J. and A. Skripchenko (2003). "Investigation of photosensitizing dyes for pathogen reduction in red cell suspensions." Biotech Histochem **78**(3-4): 171-177.

Despite recent advances in blood safety by careful donor selection and implementation of infectious disease testing, transmission of viruses, bacteria and parasites by transfusion can still rarely occur. One approach to reduce the residual risk from currently tested pathogens and to protect against the emergence of new ones is to investigate methods for pathogen inactivation. The use of photosensitizing dyes for pathogen inactivation has been studied in both red cell and platelet blood components. Optimal properties of sensitizing dyes for use in red cell suspensions include selection of dyes that traverse cell and viral membranes, bind to nucleic acids, absorb light in the red region of the spectrum, inactivate a wide range of pathogens, produce little red cell photodamage from dye not bound to nucleic acid and do not hemolyze red cells in the dark. Early research at the American Red Cross focused on the use of a class of dyes with rigid structures, such as the phenothiazine dyes, beginning with the prototypical sensitizer methylene blue. Results revealed that methylene blue phototreatment could inactivate extracellular virus, but resulted in undesirable defects in the red cell membrane that resulted in enhanced hemolysis that became evident during extended refrigerated blood storage. In addition, methylene blue phototreatment could neither inactivate intracellular viruses nor appreciably inactivate bacteria under conditions of extracellular viral killing. Attempts to improve intracellular viral inactivation led to the investigations of more hydrophobic phenothiazines, such as methylene violet or dimethylmethylene blue. Although these dyes could inactivate intracellular virus, problems with increased red cell membrane damage and hemolysis persisted or increased. Further studies using red cell additive storage solutions containing high levels of the impermeable ion, citrate, to protect against colloidal osmotic hemolysis as well as competitive inhibitors to limit sensitizer binding to red cell membranes revealed that photoinduced hemolysis stemmed from dye bound to the red cell membrane as well as dye free in solution. Use of red cell additive solutions to prevent colloidal-osmotic hemolysis and use of novel flexible dyes that only act as sensitizers when bound to their targets are two techniques that currently are under investigation for reducing red cell damage. Ultimately, the decision to implement a photodynamic method for pathogen reduction will be determined by weighing the risks of unintended adverse consequences of the procedure itself, such as the potential for genotoxicity and allergic reactions, against the cost and benefits of its implementation.

TOXNET

TOXNET compiles results from 18 database sources: CCRIS, CPDB, ChemIDplus Lite, ChemIDplus Advanced, DART/ETIC, Dietary Supplements Labels Database, DIRLINE, Drug Information Portal, GENE-TOX, Haz-Map, Household Products, HSDB, IRIS, ITER, LactMed, TOXLINE, TOXMAP, and TRI.

Years of coverage: TOXLINE and DART indicate 1965 to present, with some from as far back as the 1940's. Other TOXNET databases do not publicize years of coverage.

TOXNET was searched on Sept. 27, 2013 by Megan Goucher, Toxicology & Environmental Research and Consulting (TERC) of The Dow Chemical Company.

Terms used in the search:

Butoxyethanol, 111-76-2, MOA, Hemolysis, Alkoxyacetic Acid, Hemolysis, Carcinogenicity, Genotoxicity, Kupffer cells

Limits applied:

Publication years: 2000 - Present

The search resulted in 20 unique hits.

1. Anonymous (2001). "N,N-Dimethylformamide." Concise International Chemical Assessment Document **31**(2001).

This CICAD on N,N-dimethylformamide (DMF) was prepared jointly by the Environmental Health Directorate of Health Canada and the Commercial Chemicals Evaluation Branch of Environment Canada based on documentation prepared concurrently as part of the Priority Substances Program under the Canadian Environmental Protection Act (CEPA). The objective of assessments on Priority Substances under CEPA is to assess potential effects of indirect exposure in the general environment on human health as well as environmental effects. Occupational exposure was not addressed in this source document. Data identified as of the end of September 1999 (environmental effects) and February 2000 (human health effects) were considered in this review. Information on the nature of the peer review and availability of the source document is presented in Appendix 1. Other reviews that were also consulted include IARC (1999) and BUA (1994). Information on the peer review of this CICAD is presented in Appendix 2. This CICAD was approved as an international assessment at a meeting of the Final Review Board, held in Helsinki, Finland, on 26-29 June 2000. Participants at the Final Review Board meeting are presented in Appendix 3. The International Chemical Safety Card (ICSC 0457) for N,N-dimethylformamide, produced by the International Programme on Chemical Safety (IPCS, 1999), has also been reproduced in this document. N,N-Dimethylformamide (CAS No. 68-12-2) is an organic solvent produced in large quantities throughout the world. It is used in the chemical industry as a solvent, an intermediate, and an additive. It is a colourless liquid with a faint amine odour. It is completely miscible with water and most organic solvents and has a relatively low vapour pressure. When emitted into air, most of the DMF

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released remains in that compartment, where it is degraded by chemical reactions with hydroxyl radicals. Indirect releases of DMF to air, such as transfers from other environmental media, play only a small role in maintaining levels of DMF in the atmosphere. DMF in air is estimated to be photooxidized over a period of days. However, some atmospheric DMF can reach the aquatic and terrestrial environment, presumably during rain events. When DMF is released into water, it degrades there and does not move into other media. When releases are into soil, most of the DMF remains in the soil - presumably in soil pore water - until it is degraded by biological and chemical reaction. Releases to water or soil are expected to be followed by relatively rapid biodegradation (half-life 18-36 h). If DMF reaches groundwater, its anaerobic degradation will be slow. The use pattern of DMF is such that exposure of the general population is probably very low. Since most DMF appears to be released to air in the sample country, and based on the fate of DMF in the ambient environment, biota are expected to be exposed to DMF primarily in air; little exposure to DMF from surface water, soil, or benthic organisms is expected. Based on this, and because of the low toxicity of DMF to a wide range of aquatic and soil organisms, the focus of the environmental risk characterization is terrestrial organisms exposed directly to DMF in ambient air. DMF is readily absorbed following oral, dermal, or inhalation exposure. Following absorption, DMF is uniformly distributed, metabolized primarily in the liver, and relatively rapidly excreted as metabolites in urine. The major pathway involves the hydroxylation of methyl moieties, resulting in N-(hydroxymethyl)-N-methylformamide (HMMF), which is the major urinary metabolite in humans and animals. HMMF in turn can decompose to N-methylformamide (NMF). In turn, enzymatic N-methyl oxidation of NMF can produce N-(hydroxymethyl)formamide (HMF), which further degenerates to formamide. An alternative pathway for the metabolism of NMF is oxidation of the formyl group, resulting in N-acetyl-S-(N-methylcarbamoyl)cysteine (AMCC), which has been identified as a urinary metabolite in rodents and humans. A reactive intermediate, the structure of which has not yet been determined (possibly methyl isocyanate), is formed in this pathway; while direct supporting experimental evidence was not identified, this intermediate is suggested to be the putatively toxic metabolite. Available data indicate that a greater proportion of DMF may be metabolized by the putatively toxic pathway in humans than in experimental animals. There is metabolic interaction between DMF and alcohol, which, though not well understood, may be due, at least in part, to its inhibitory effect on alcohol dehydrogenase. Consistent with the results of studies in experimental animals, available data from case reports and cross-sectional studies in occupationally exposed populations indicate that the liver is the target organ for the toxicity of DMF in humans. The profile of effects is consistent with that observed in experimental animals, with gastrointestinal disturbance, alcohol intolerance, increases in serum hepatic enzymes (aspartate aminotransferase, alanine aminotransferase, γ -glutamyl transpeptidase, and alkaline phosphatase), and histopathological effects and ultrastructural changes (hepatocellular necrosis, enlarged Kupffer cells, microvesicular steatosis, complex lysosomes, pleomorphic mitochondria, and fatty changes with occasional lipogranuloma) being observed. Based on the limited data available, there is no convincing, consistent evidence of increases in tumours at any site associated with exposure to DMF in the occupational environment. Case reports of testicular cancers have not been confirmed in a cohort and case-control study. There have been no consistent increases in tumours at other sites associated with exposure to DMF. There is also little consistent, convincing evidence of genotoxicity in populations

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occupationally exposed to DMF, with results of available studies of exposed workers (to DMF and other compounds) being mixed. The pattern of observations is not consistent with variations in exposure across studies. However, in view of the positive dose-response relationship observed in the one study in which it was investigated, this area may be worthy of additional work, although available data on genotoxicity in experimental systems are overwhelmingly negative. DMF has low acute toxicity and is slightly to moderately irritating to the eyes and skin. No data were identified regarding the sensitization potential of DMF. In acute and repeated-dose toxicity studies, DMF has been consistently hepatotoxic, inducing effects on the liver at lowest concentrations or doses. The profile of effects includes alterations in hepatic enzymes characteristic of toxicity, increases in liver weight, progressive degenerative histopathological changes and eventually cell death, and increases in serum hepatic enzymes. A dose-response has been observed for these effects in rats and mice following inhalation and oral exposure. Species variation in sensitivity to these effects has been observed, with the order of sensitivity being mice > rats > monkeys. Although the database for carcinogenicity is limited to two adequately conducted bioassays in rats and mice, there have been no increases in the incidence of tumours following chronic inhalation exposure to DMF. The weight of evidence for genotoxicity is overwhelmingly negative, based on extensive investigation in in vitro assays, particularly for gene mutation, and a more limited database in vivo. In studies with laboratory animals, DMF has induced adverse reproductive effects only at concentrations greater than those associated with adverse effects on the liver, following both inhalation and oral exposure. Similarly, in well conducted and reported primarily recent developmental studies, fetotoxic and teratogenic effects have been consistently observed only at maternally toxic concentrations or doses. Available data are inadequate as a basis for assessment of the neurological or immunological effects of DMF. The focus of this CICAD and the sample risk characterization is primarily effects of indirect exposure in the general environment. Air in the vicinity of point sources appears to be the greatest potential source of exposure of the general population to DMF. Based on the results of epidemiological studies of exposed workers and supporting data from a relatively extensive database of investigations in experimental animals, the liver is the critical target organ for the toxicity of DMF. A tolerable concentration of 0.03 ppm (0.1 mg/m³) has been derived on the basis of increases in serum hepatic enzymes. Data on the toxicity of DMF to terrestrial vascular plants have not been identified. Effect concentrations for indicators of the potential sensitivities of trees, shrubs, and other plants are high; hence, it is unlikely that terrestrial plants are particularly sensitive to DMF. For other terrestrial organisms, an estimated no-effects value of 15 mg/m³ has been derived based on a critical toxicity value for hepatic toxicity in mice divided by an application factor. Comparison of this value with a conservative estimated exposure value indicates that it is unlikely that DMF causes adverse effects on terrestrial organisms in the sample country. KW - < ANIMAL >

2. Anonymous (2003). "Final Report on the Safety Assessment of Stearoxy Dimethicone, Dimethicone, Methicone, Amino Bispropyl Dimethicone, Aminopropyl Dimethicone, Amodimethicone, Amodimethicone Hydroxystearate." *Int J Toxicol* **2**(2003): 11-35.

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Dimethicone is a fluid mixture of fully methylated linear siloxane polymers end-blocked with trimethylsiloxy units. Methicone is a linear monomethyl polysiloxane. The other dimethicones and methicones covered in this review are siloxane polymers of Dimethicone and Methicone. Most of the data re-viewed in this report are studies of Dimethicone. Almost all of the 20 ingredients function as conditioning agents in cosmetic formulations. FDA reported seven of the ingredients used in 1998 in a total of 1884 formulations; CTFA reported 10 uses. The highest current concentration of use was 15%. Dimethicone has both food and over-the-counter topical drug use. Its use in foods is limited by molecular weight. Clinical and animal absorption studies generally reported that Dimethicone was not absorbed following oral or dermal exposure, although some absorption was seen in humans following ingestion of a Dimethicone sample containing low-molecular-weight polymers. Dimethicone, Methicone, and Vinyl dimethicone were not acutely toxic following oral exposure. Mice and rats were dosed for 90 days with up to 10% Dimethicone without adverse effect. Changes in body weight or spleen weight were observed in some rat studies. Anal leakage was noted when Dimethicone fluids of low viscosity were used. Bile deposits in the Kupffer and hepatic cells were observed in dogs dosed with 3 g/kg/day for 6 months. The dermal LD50 for Dimethicone was > 2 g/kg in rats and rabbits. The dermal LD50 for Methicone was > 20 ml/kg in rabbits. The dermal LD50 for Vinyl dimethicone was > 16 ml/kg in rabbits. No adverse reactions were found in rabbits following short-term dermal dosing with 6% to 79% Dimethicone. Adverse effects were noted with a hand cream formulation containing 1% Dimethicone (the other components of the cream were not disclosed). Only limited inhalation toxicity data were available. A "200 fluid" did produce adverse effects in one study. Methicone and Vinyl dimethicone were negative in acute exposure studies using rats. Hexyl Methicone did produce toxic effects in Fischer F344/N rats-the LC50 was 1.8 mg/L. Most dermal irritation studies using rabbits classified Dimethicone as a minimal irritant. Studies that scored reactions according to the Draize scale reported PIs of ≤ 2.8 (with test samples containing 5% to 100% Dimethicone). Dimethicone (tested undiluted and at 79%) was not a sensitizer in four assays using mice and guinea pigs. It was not a sensitizer at 5.0% in a clinical RIPT using 83 panelists. Vinyl dimethicone was not irritating to rabbits following a 4-h exposure. Most ocular irritation studies using rabbits classified Dimethicone as a mild to minimal irritant. The most common finding was a conjunctival reaction. However, a few studies reported severe reactions. Similar to Dimethicone, Methicone and Vinyl dimethicone also produced conjunctival reactions. Dimethicone was tested in numerous oral-dose (using rats) and dermal-dose (using rats, rabbits, monkeys) reproductive and developmental toxicity studies. In a few studies, treated males had significantly decreased body weight and/or decreased testes or seminal vesicles weights. No treatment-related adverse findings were noted in dosed pregnant females or fetuses. Dimethicone was negative in all mutagenicity assays. It was negative in both an oral (tested at 91%) and dermal (tested an unknown concentration) dose carcinogenicity assay using mice. Discussion. The CIR Expert Panel considered it unlikely that any of these polymers would be significantly absorbed into the skin due to the large molecular weight of these polymers. Inhalation exposure, however, was of concern given the limited inhalation toxicity findings in the report. It was noted, however, that only a few of these ingredients are used in aerosol formulations and at a very low concentration. In addition, the Panel was informed that particles from cosmetic formulations containing these ingredients would not likely be inhaled. In particular, it was stated that expected particle sizes would primarily be in the range of 60 to 80

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microns, and less than 1 % would be under 10 microns, which is an upper limit for respirable particles. The Panel expects that the manufacture process for cosmetic formulations in which these ingredients are found and which may be inhaled would continue to produce particle size distributions that are not significantly respirable. Overall, the safety test data in the report support the safety of these ingredients at the concentrations that they are known to be used in cosmetic formulations. Accordingly, the CIR Expert Panel was of the opinion that Stearoxy Dimethicone, Dimethicone, Methicone, Amino Bispropyl Dimethicone, Amino-propyl Dimethicone, Amodimethicone, Amodimethicone Hydroxystearate, Behenoxy Dimethicone, C24-28 Alkyl Methicone, C30-45 Alkyl Methicone, C30-45 Alkyl Dimethicone, Cetearyl Methicone, Cetyl Dimethicone, Dimethoxysilyl Ethylenediaminopropyl Dimethicone, Hexyl Methicone, Hydroxypropyldimethicone, Stearamidopropyl Dimethicone, Stearyl Dimethicone, Stearyl Methicone, and Vinyl dimethicone may be used safely in cosmetic formulations. Conclusion. Based on the available data, the CIR Panel concludes that Stearoxy Dimethicone, Dimethicone, Methicone, Amino Bis-propyl Dimethicone, Aminopropyl Dimethicone, Amodimethicone, Amodimethicone Hydroxystearate, Behenoxy Dimethicone, C24-28 Alkyl Methicone, C30-45 Alkyl Methicone, C30-45 Alkyl Dimethicone, Cetearyl Methicone, Cetyl Dimethicone, Dimethoxysilyl Ethylenediaminopropyl Dimethicone, Hexyl Methicone, Hydroxypropyldimethicone, Stearamidopropyl Dimethicone, Stearyl Dimethicone, Stearyl Methicone, and Vinyl dimethicone are safe as used in cosmetic products.

3. Anonymous (2003). "Nitrobenzene." Environmental Health Criteria **230**(2003).

Effects on laboratory mammals and in vitro test systems Nitrobenzene causes toxicity in multiple organs by all routes of exposure. Methaemoglobinaemia results from oral, dermal, subcutaneous and inhalational nitrobenzene exposure in mice and rats, with consequent haemolytic anaemia, splenic congestion and liver, bone marrow and spleen haematopoiesis. Splenic capsular lesions were seen in rats by both gavage (at doses as low as 18.75 mg/kg of body weight per day) and dermal (at 100 mg/kg of body weight per day and above) routes of administration. Similar splenic lesions have previously been observed with aniline-based dyes, some of which produced splenic sarcomas in chronic carcinogenicity studies in rats. Effects on the liver were noted in mice and rats after both gavage and dermal administration of nitrobenzene, with centrilobular hepatocyte necrosis, hepatocellular nucleolar enlargement, severe hydropic degeneration and pigment accumulation in Kupffer cells reported. Increased vacuolation of the X-zone of the adrenal gland was noted in female mice after oral and dermal dosing. In subchronic oral and dermal studies in mice and rats, central nervous system lesions in the cerebellum and brain stem were life-threatening. These lesions, including petechial haemorrhages, may be direct toxic effects or mediated by vascular effects of hypoxia or hepatic toxicity. Depending on the dose, these neurotoxic effects were grossly apparent as ataxia, head-tilt and arching, loss of righting reflex, tremors, coma and convulsions. Other target organs included kidney (increased weight, glomerular and tubular epithelial swelling, pigmentation of tubular epithelial cells), nasal epithelium (glandularization of the respiratory epithelium, pigment deposition in and degeneration of olfactory epithelium), thyroid (follicular cell hyperplasia), thymus (involution) and pancreas (mononuclear cell infiltration), while lung pathology (emphysema, atelectasis

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and bronchiolization of alveolar cell walls) was reported in rabbits. The potential carcinogenicity and toxicity of inhaled nitrobenzene were evaluated following long-term exposure (505 days) of male and female B6C3F1 mice, male and female Fischer-344 rats and male Sprague-Dawley rats. Survival was not adversely affected at the concentrations tested (up to 260 mg/m³ [50 ppm] for mice; up to 130 mg/m³ [25 ppm] for rats), but inhaled nitrobenzene was toxic and carcinogenic in both species and both rat strains, inducing a spectrum of benign and malignant (lung, thyroid, mammary gland, liver, kidney) neoplasias. Nitrobenzene was non-genotoxic in bacteria and mammalian cells in vitro and in mammalian cells in vivo. Studies reported included DNA damage and repair assays, gene mutation assays, chromosomal effects assays and cell transformation assays. Numerous studies have confirmed that nitrobenzene is a testicular toxicant, with the most sensitive spermatogenic end-points being sperm count and motility, followed by progressive motility, viability, presence of abnormal sperm and, finally, the fertility index. In a two-generation reproductive toxicity study in Sprague-Dawley rats by the inhalational route, nitrobenzene at 200 mg/m³ (40 ppm), but not at 5 or 51 mg/m³ (1 or 10 ppm), caused a large decrease in the fertility index of F0 and F1 generations, associated with male reproductive system toxicity; this decreased fertility was partially reversible, when the F1 generation from the 200 mg/m³ group was mated with virgin untreated females after a 9-week recovery period. However, in an oral dosing study in the same rat strain (20-100 mg/kg of body weight from 14 days pre-mating to day 4 of lactation), while pup body weight was lowered and postnatal loss was increased, nitrobenzene was without effect on reproductive parameters. The lack of effect on fertility in this study was due to the short pre-mating dosage interval and the fact that rats produce sperm in very large excess. Impaired male fertility with significant testicular atrophy was seen in mice and rats; effects in mice were apparent at gavage doses of 300 mg/kg of body weight per day and dermal doses of 800 mg/kg of body weight per day and in rats at gavage doses of 75 mg/kg of body weight per day and dermal doses of 400 mg/kg of body weight per day. Testicular toxicity was seen as desquamation of the seminiferous epithelium, the appearance of multinucleated giant cells, gross atrophy and prolonged aspermia. Nitrobenzene has direct effects on the testis, shown by in vivo and in vitro studies. Spermatogenesis is affected, with exfoliation of predominantly viable germ cells and degenerating Sertoli cells. The main histopathological effects are degenerated spermatocytes. In general, maternal reproductive organs were not affected, except for one study where uterine atrophy was seen in mice after a dermal dose of 800 mg/kg of body weight per day. Developmental toxicity studies in rats and rabbits indicated that inhalation exposure to nitrobenzene did not result in fetotoxic, embryo-toxic or teratogenic effects at concentrations sufficient to produce maternal toxicity. At the highest concentration tested in these studies (530 mg/m³ [104 ppm] in a rabbit study), the mean numbers of resorption sites and percentage of resorptions/implants were higher in this group than in concurrent controls, but were within the historical control range; maternal effects (i.e., increased methaemoglobin levels and increased liver weights) were noted from 210 mg/m³ (41 ppm). In a study on the immunotoxicity of nitrobenzene in B6C3F1 mice, nitrobenzene caused increased cellularity of the spleen, a degree of immunosuppression (diminished IgM response to sheep red blood cells) and bone marrow stimulation. Host resistance to microbial or viral infection was not markedly affected by nitrobenzene, although there was a trend towards increased susceptibility in cases in which T-cell function contributed to host defence. Effects on humans Nitrobenzene is toxic to humans by

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inhalational, dermal and oral exposure. The main systemic effect associated with human exposure to nitrobenzene is methaemoglobinaemia. Numerous accidental poisonings and deaths in humans from ingestion of nitrobenzene have been reported. In cases of oral ingestion or in which the patients were apparently near death due to severe methaemoglobinaemia, termination of exposure and prompt medical intervention resulted in gradual improvement and recovery. Although human exposure to sufficiently high quantities of nitrobenzene can be lethal via any route of exposure, it is considered unlikely that levels of exposure high enough to cause death would occur except in cases of industrial accidents or suicides. The spleen is likely to be a target organ during human exposure to nitrobenzene; in a woman occupationally exposed to nitrobenzene in paint (mainly by inhalation), the spleen was tender and enlarged. Liver effects, including hepatic enlargement and tenderness and altered serum chemistries, have been reported in a woman inhalationally exposed to nitrobenzene. Neurotoxic symptoms reported in humans after inhalation exposure to nitrobenzene have included headache, confusion, vertigo and nausea. Effects in orally exposed persons have also included those symptoms, as well as apnoea and coma. Effects on organisms in the environment Nitrobenzene appears to be toxic to bacteria and may adversely affect sewage treatment facilities if present in high concentrations in influent. The lowest toxic concentration reported for microorganisms is for the bacterium *Nitrosomonas*, with an EC50 of 0.92 mg/litre based upon the inhibition of ammonia consumption. Other reported values are a 72-h no-observed-effect concentration (NOEC) of 1.9 mg/litre for the protozoan *Entosiphon sulcatum* and an 8-day lowest-observed-effect concentration (LOEC) of 1.9 mg/litre for the blue-green alga *Microcystis aeruginosa*. For freshwater invertebrates, acute toxicity (24- to 48-h LC50 values) ranged from 24 mg/litre for the water flea (*Daphnia magna*) to 140 mg/litre for the snail (*Lymnaea stagnalis*). For marine invertebrates, the lowest acute toxicity value reported was a 96-h LC50 of 6.7 mg/litre for the mysid shrimp (*Mysidopsis bahia*). The lowest chronic test value reported was a 20-day NOEC of 1.9 mg/litre for *Daphnia magna*, with an EC50, based on reproduction, of 10 mg/litre. Freshwater fish showed similar low sensitivity to nitrobenzene. The 96-h LC, values ranged from 24 mg/litre for the medaka (*Oryzias latipes*) to 142 mg/litre for the guppy (*Poecilia reticulata*). There was no effect on mortality or behaviour of medaka at 7.6 mg/litre over an 18-day exposure. Hazard and risk evaluation

Methaemoglobinaemia and subsequent haematological and splenic changes have been observed in exposed humans, but the data do not allow quantification of the exposure-response relationship. In rodents, methaemoglobinaemia, haematological effects, testicular effects and, in the inhalation studies, effects on the respiratory system were found at the lowest doses tested. Methaemoglobinaemia, bilateral epididymal hypospermia and bilateral testicular atrophy were observed at the lowest exposure level studied, 5 mg/m³ (1 ppm), in rats. In mice, there was a dose-related increase in the incidence of bronchiolization of alveolar walls and alveolar/bronchial hyperplasia at the lowest dose tested of 26 mg/m³ (5 ppm). Carcinogenic response was observed after exposure to nitrobenzene in rats and mice: mammary adenocarcinomas were observed in female B6C3F1 mice, and liver carcinomas and thyroid follicular cell adenocarcinomas were seen in male Fischer-344 rats. Benign tumours were observed in five organs. Studies on genotoxicity have usually given negative results. Although several metabolic products of nitrobenzene are candidates for cancer causality, the mechanism of carcinogenic action is not known. Because of the likely commonality of redox mechanisms in test animals and humans, it is hypothesized that nitrobenzene may

cause cancer in humans by any route of exposure. Exposure of the general population to nitrobenzene from air or drinking-water is likely to be very low. Although no no-observed-adverse-effect level (NOAEL) could be derived from any of the toxicological studies, there is a seemingly low risk for non-neoplastic effects. If exposure values are low enough to avoid non-neoplastic effects, it is expected that carcinogenic effects will not occur. Acute poisonings by nitrobenzene in consumer products have occurred frequently in the past. Significant human exposure is possible, due to the moderate vapour pressure of nitrobenzene and extensive skin absorption. Furthermore, the relatively pleasant almond smell of nitrobenzene may not discourage people from consuming food or water contaminated with it. Infants are especially susceptible to the effects of nitrobenzene. There is limited information on exposure in the workplace. In one workplace study, exposure concentrations were of the same order of magnitude as the lowest-observed-adverse-effect levels (LOAELs) in a long-term inhalation study. Therefore, there is significant concern for the health of workers exposed to nitrobenzene. Nitrobenzene shows little tendency to bioaccumulate and appears to undergo both aerobic and anaerobic biotransformation. For terrestrial systems, the levels of concern reported in laboratory tests are unlikely to occur in the natural environment, except possibly in areas close to nitrobenzene production and use and areas contaminated by spillage. Using the available acute toxicity data and a statistical distribution method, together with an acute:chronic toxicity ratio derived from data on crustaceans, the concentration limit for nitrobenzene to protect 95% of freshwater species with 50% confidence may be estimated to be 200 µg/litre. Nitrobenzene is thus unlikely to pose an environmental hazard to aquatic species at levels typically reported in surface waters, around 0.1-1 µg/litre. Even at highest reported concentrations (67 µg/litre), nitrobenzene is unlikely to be of concern to freshwater species. There is not enough information to derive a guideline value for marine organisms.

4. Burgi, H., et al. (2001). "The toxicology of iodate: a review of the literature." Thyroid. **11**(5): 449-456. [Thyroid : official journal of the American Thyroid Association].

Because it is more stable than iodide, most health authorities preferentially recommend iodate as an additive to salt for correcting iodine deficiency. Even though this results in a low exposure of at most 1,700 µg/d, doubts have recently been raised whether the safety of iodate has been adequately documented. In humans and rats, oral bioavailability of iodine from iodate is virtually equivalent to that from iodide. When given intravenously to rats, or when added to whole blood or tissue homogenates in vitro or to foodstuff, iodate is quantitatively reduced to iodide by nonenzymatic reactions, and thus becomes available to the body as iodide. Therefore, except perhaps for the gastrointestinal mucosa, exposure of tissues to iodate might be minimal. At much higher doses given intravenously (i.e., above 10 mg/kg), iodate is highly toxic to the retina. Ocular toxicity in humans has occurred only after exposure to doses of 600 to 1,200 mg per individual. Oral exposures of several animal species to high doses, exceeding the human intake from fortified salt by orders of magnitude, pointed to corrosive effects in the gastrointestinal tract, hemolysis, nephrotoxicity, and hepatic injury. The studies do not meet current standards of toxicity testing, mostly because they lacked toxicokinetic data and did not separate iodate-specific effects from the effects of an overdose of any form of iodine. With regard to tissue injury, however, the data indicate a negligible risk of the small oral long-term doses achieved with iodate-

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fortified salt. Genotoxicity and carcinogenicity data for iodate are scarce or nonexistent. The proven genotoxic and carcinogenic effects of bromate raise the possibility of analogous activities of iodate. However, iodate has a lower oxidative potential than bromate, and it did not induce the formation of oxidized bases in DNA under conditions in which bromate did, and it may therefore present a lower genotoxic and carcinogenic hazard. This assumption needs experimental confirmation by proper genotoxicity and carcinogenicity data. These in turn will have to be related to toxicokinetic studies, which take into account the potential reduction of iodate to iodide in food, in the intestinal lumen or mucosa, or eventually during the liver passage.

5. Gad, S. C., et al. (2012). Evaluation of the toxicity of intravenous delivery of auroshell particles (gold-silica nanoshells). Int J Toxicol. **31**: 584-594. [International journal of toxicology].

Gold nanoshells (155 nm in diameter with a coating of polyethylene glycol 5000) were evaluated for preclinical biocompatibility, toxicity, and biodistribution as part of a program to develop an injectable device for use in the photothermal ablation of tumors. The evaluation started with a complete good laboratory practice (GLP) compliant International Organization for Standardization (ISO)-10993 biocompatibility program, including cytotoxicity, pyrogenicity (US Pharmacopeia [USP] method in the rabbit), genotoxicity (bacterial mutagenicity, chromosomal aberration assay in Chinese hamster ovary cells, and in vivo mouse micronucleus), in vitro hemolysis, intracutaneous reactivity in the rabbit, sensitization (in the guinea pig maximization assay), and USP/ISO acute systemic toxicity in the mouse. There was no indication of toxicity in any of the studies. Subsequently, nanoshells were evaluated in vivo by intravenous (iv) infusion using a trehalose/water solution in a series of studies in mice, Sprague-Dawley rats, and Beagle dogs to assess toxicity for time durations of up to 404 days. Over the course of 14 GLP studies, the gold nanoshells were well tolerated and, when injected iv, no toxicities or bioincompatibilities were identified.

6. Ghosh, M., et al. (2013). "Cytotoxic, genotoxic and the hemolytic effect of titanium dioxide (TiO₂) nanoparticles on human erythrocyte and lymphocyte cells in vitro." J Appl Toxicol. **33**(10): 1097-1110. [Journal of applied toxicology : JAT].

With the increasing clinical use of titanium dioxide (TiO₂) nanoparticles, a better understanding of their safety in the blood stream is required. The present study evaluates the toxic effect of commercially available TiO₂ nanoparticles (~100 nm) using a battery of cytotoxic, genotoxic, hemolytic and morphological parameters. The cytotoxic effects of TiO₂ nanoparticles in human lymphocyte cells were studied with respect to membrane damage, mitochondrial function, metabolic activity and lysosomal membrane stability. Genotoxicity in lymphocyte cells was quantitated using a comet assay. The mode of cell death (apoptosis/necrosis) was evaluated using PI/Annexin V staining. TiO₂ nanoparticles were also evaluated for their hemolytic properties, osmotic fragility and interaction with hemoglobin. Human erythrocyte cells were studied for morphological alterations using atomic force microscopy (AFM). Results suggest that the particles could induce a significant reduction in mitochondrial dehydrogenase activity in human lymphocyte cells. Membrane integrity remained unaffected by nanoparticle

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treatment. DNA damage and apoptosis were induced by TiO₂ nanoparticles in a dose-dependent manner. A study on human erythrocyte cells revealed a hemolytic property of TiO₂ nanoparticles characterized by spherocytosis and echinocytosis. Spectral analysis revealed a hemoglobin TiO₂ nanoparticle interaction. Our in vitro study results suggest that commercially available blood contacting nanoparticles (TiO₂ nanoparticle) should be carefully evaluated for their toxic potential. Copyright © 2013 John Wiley & Sons, Ltd.

7. Hadba, A. R., et al. (2011). "Isocyanate-functional adhesives for biomedical applications. Biocompatibility and feasibility study for vascular closure applications." J Biomed Mater Res B Appl Biomater. **99**(1): 27-35. [Journal of biomedical materials research. Part B, Applied biomaterials].

Biodegradable isocyanate-functional adhesives based on poly(ethylene glycol)-adipic acid esters were synthesized, characterized, and evaluated in vitro and in vivo. Two types of formulations, P2TT and P2MT, were developed by functionalization with 2,4-tolylene diisocyanate (TDI) or 4,4'-methylene-bis(phenyl isocyanate) (MDI), respectively, and branching with 1,1,1-trimethylolpropane (TMP). The biocompatibility of the synthesized adhesive formulations was evaluated as per ISO 10993. Cytotoxicity, systemic toxicity, pyrogenicity, genotoxicity (reverse mutation of *Salmonella typhimurium* and *Escherichia coli*), hemolysis, intracutaneous reactivity, and delayed-type hypersensitivity were evaluated. All formulations met the requirements of the conducted standard tests. The biological behavior and ability of the adhesive formulations to close an arteriotomy and withstand arterial pressure following partial approximation with a single suture were evaluated in a rat abdominal aorta model. Animals were evaluated at 1, 2, 3, and 4 weeks after surgery. Macroscopic and histopathologic evaluation of explanted arteries suggested that the P2TT formulation had better in vivo performance than the P2MT formulation. Additionally, the P2TT formulation resulted in less tissue reaction than P2MT formulation. To our knowledge, this is the first study demonstrating the potential of this new class of isocyanate-functional degradable adhesives for vascular applications.

8. Hecker, S. J. and M. J. Miller (2012). Molecular Archaeology: Vintage Beta Lactams for Resistant Gram Negative Infectio. RePORTER Database National Institutes of Health.

DESCRIPTION (provided by applicant): We propose that existing beta-lactam scaffolds can be modified to address the emerging threat of Gram- negative resistance. A wealth of beta-lactams were synthesized from the 1950s into the 1980s but were never developed into antimicrobial drugs. Under the current stress of increasing drug resistance, and with decades of accrued medicinal chemistry knowledge at our fingertips, certain of these molecules are of renewed interest as a result of their non-susceptibility to resistance mechanisms that have since evolved. The utility of beta-lactam antibiotics has been compromised by the spread of beta-lactamase enzymes, both of the serine type (Classes A, C and D) and the metallo type (Class B). The carbapenems, while stable to most Class A and Class C beta-lactamases, are susceptible to Class B enzymes as well as emerging *Klebsiella pneumoniae* carbapenemase (KPC) Class A enzymes. Monobactams, by contrast, are intrinsically stable to Class B

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metalloenzymes, and we have synthesized a prototype compound that is additionally stable to KPC enzymes. A new agent that is not susceptible to any of these enzymes would have a major impact on restoring the utility of the beta-lactam class. We have evaluated numerous existing, older beta-lactam agents against panels of bacteria that are well-characterized with respect to expression of beta-lactamase enzymes as well as efflux pumps. The Structure-Activity Relationships (SAR) from this data set have enabled us to design several series of new beta-lactam molecules that are expected to retain potent activity against organisms that are resistant to currently-available agents. We hypothesize that we can modify a carefully selected scaffold that is active against pathogens producing KPC enzymes to include moieties that would make it potent against Class A and Class C beta-lactamases. To accomplish this, we propose two aims in the exploratory R21 phase of the project to provide us with our drug candidate. Then we propose three development aims in the R33 phase. The aims for the R21 phase include: Aim 1. Optimize the in vitro activity of the selected lead compounds with respect to spectrum and potency. Aim 2. Conduct pharmacokinetics (PK) studies and optimize efficacy in infection models. Aims to be conducted during R33 phase include: Aim 3. Conduct higher order pharmacokinetic studies. Aim 4. Conduct in vitro toxicology battery and pilot toxicology studies. Aim 5. Conduct 14-day range-finding toxicology studies. Upon successful completion of this project, we will be ready to proceed into IND-enabling toxicology studies. PUBLIC HEALTH RELEVANCE: Monobactams are penicillin-like drugs that were used to treat bacterial illness in the past. Though their development lagged with the advent of new classes of antibiotics, emerging drug-resistance to such newer classes has renewed our interest in monobactams. We propose to take an older monobactam scaffold and modify it using more recently developed chemistry to create a novel drug that can address difficult, drug-resistant bacterial infections.

9. Joray, M. B., et al. (2011). Antibacterial activity of the plant-derived compounds 23-methyl-6-O-desmethyllauricepyrone and (Z,Z)-5-(trideca-4,7-dienyl)resorcinol and their synergy with antibiotics against methicillin-susceptible and -resistant *Staphylococcus aureus*. *J Agric Food Chem*, **59**: 11534-11542. [Journal of agricultural and food chemistry].

The present study investigated the antibacterial activity of two plant-derived compounds, 23-methyl-6-O-desmethyllauricepyrone (1) and (Z,Z)-5-(trideca-4,7-dienyl)resorcinol (2), and their synergistic effects with erythromycin and gentamicin against methicillin-susceptible (MSSA) and gentamicin- and methicillin-resistant *Staphylococcus aureus* (MRSA). Studies of the individual antibacterial activity of each plant-derived compound and synergy experiments were carried out, by the microdilution test in agar and by the checkerboard method, respectively. Compound 1 showed minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of 2 and 8 $\mu\text{g/mL}$, respectively, against both strains of *S. aureus*, while compound 2 exhibited anti-MSSA and anti-MRSA activity with MICs and MBCs of 4 and 8 and 2 and 8 $\mu\text{g/mL}$, respectively. Time-kill curves showed that, while compound 1 produced complete killing of both strains at 24 h from the beginning of the experiment, 2 produced the same effect in the first hour. Combinations of 1 with erythromycin or gentamicin showed a notable synergism against MSSA, which enabled

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the antibiotic concentration to decrease by up to 300 or 260 times, respectively. When the aminoglycoside was placed together with compound 2, only an additive effect was observed. The assayed compounds did not produce erythrocyte hemolysis or genotoxicity and they did not affect macrophage viability at the effective or higher concentrations. These results suggest that both compounds could be considered as promising antibacterial agents while compound 1 could be used in combinatory therapies with erythromycin and gentamicin.

10. Klaunig, J. E. (2006). "Epigenetic Mechanisms Of Hemangiosarcoma Induction." Toxicol Sci **90**(1-5): 67-68.

In the mouse liver, chronic exposure to selective agents results in the induction of hemangiosarcomas. Several of these agents, including the solvent 2-Butoxyethanol, are negative for DNA reactivity in standard genotoxicity bioassays. Unlike the rat, the male and female mouse (B6C3F1) display a historical background rate of liver hemangiosarcomas of 2.5% and 0.8% respectively. In the case of 2-Butoxyethanol, concomitant with the formation of hemangiosarcomas, was the induction of hemolysis and an increase in hemosiderin deposition in Kupffer cells. Studies in our laboratory have been directed at understanding the mode of action of the hemangiosarcomas induce in the liver by epigenetic mechanisms. 2-Butoxyethanol serves as one model for these studies. Treatment of B6C3F1 male mice (by gavage) with 2- Butoxyethanol in a subchronic protocol produced a dose and time dependent increase in endothelial cell proliferation in the liver. Along with the increase in endothelial cell proliferation was a similar time and dose related increase in liver oxidative damage and increased hemosiderin deposition (positive by Perls stain). Co-treatment with antioxidants prevented the increase in oxidative stress and the increase in cell proliferation while having no effect on iron deposition in the liver. A pivotal role for the Kupffer cell in the induction of the mouse hemangiosarcomas was proposed suggesting that activation of the Kupffer cells produce an increase in the production of reactive oxygen species and an increase in endothelial growth factors resulting in an increase in endothelial cell proliferation. In the mouse liver this increase in cell proliferation produces a tumor promotion effect on spontaneously present preneoplastic endothelial cells.

11. Mellert, W., et al. (2004). Aniline: early indicators of toxicity in male rats and their relevance to spleen carcinogenicity. Hum Exp Toxicol. **23**: 379-389. [Human & experimental toxicology].

Early indicators of aniline hydrochloride (AH) toxicity were investigated in male Fisher 344 rats for 1 or 4 weeks at dietary dose levels of 10, 30 or 100 mg/kg body weight (bw)/day (actual intake at least 6, 17 and 57 mg/kg). The doses were based on earlier studies that had shown spleen toxicity and carcinogenicity in male rats at 100 mg/kg/day but not at 10 mg/kg/day. In the present study a dose-related formation of haemoglobin adducts and Heinz bodies was found from 10 and 30 mg/kg bw/ day, respectively, onwards. Signs of anaemia (decreased red blood cell counts and increased reticulocytes) were recorded from 30 mg/kg onwards. At 100 mg/kg, an overt haemolytic anaemia was associated with increases in serum transferrin concentration and total iron binding capacity in the blood reflecting major perturbations in iron metabolism. At this dose there was an increase in peripheral neutrophil leucocytosis in

the blood, indicating an inflammatory process in the spleen. Histopathologic evaluation showed a focal perisplenitis and haemosiderin deposition in sinusoidal Kupffer cells of the liver at 100 mg/kg. These results corroborate the contention that carcinogenic doses of aniline cause early effects on haematological parameters, inflammatory reaction in the spleen and perturbations in iron metabolism as a result of haemolytic anaemia. Accordingly, the carcinogenicity of aniline may be linked to definable threshold-related processes.

12. Mizukami, S., et al. (2010). Tumor promotion by copper-overloading and its enhancement by excess iron accumulation involving oxidative stress responses in the early stage of a rat two-stage hepatocarcinogenesis model. Chem Biol Interact. **185**: 189-201. [Chemico-biological interactions].

To investigate liver tumor promotion mechanisms of copper (Cu)- and iron (Fe)-overloading, immunolocalization of metal-related biomolecules and lipid peroxidation end products was examined in preneoplastic liver cell foci that expressed glutathione S-transferase placental form (GST-P) in early-stage tumor promotion over 6 weeks in a rat two-stage hepatocarcinogenesis model. Gene expression and concentrations of thiobarbituric acid-reactive substance (TBARS) in the liver were also analyzed. Cu-overloading alone exerted a weak promoting activity, which was enhanced by Fe-overloading. By Cu-overloading, GST-P(+) foci that co-expressed transferrin receptors or downregulated ceruloplasmin increased, suggesting preneoplastic lesion-specific enhancement of oxidative cellular stress. Cu-overloading also increased transcripts of antioxidant enzymes (Gstm3 and Gst Yc2 subunit), cell proliferation, and numbers of single liver cells expressing GST-P or heme oxygenase-1 (HO-1) in the liver, suggesting that oxidative stress induces single-cell toxicity, with the ensuing regeneration contributing to tumor promotion. Fe-overloading increased liver TBARS and HO-1-expressing Kupffer cells, the latter suggesting protection against inflammatory stimuli causing fluctuating proinflammatory cytokine mRNA levels. By co-overloading of Cu and Fe, Cu-overload-related single liver cell toxicity and regeneration increased, as did cytokine imbalances involving increased cyclooxygenase-2-producing Kupffer cells and accumulation of malondialdehyde within GST-P(+) foci. These results suggest an involvement of oxidative stress responses in Cu-induced tumor promotion and Fe-induced enhancement by increasing cytokine imbalances and GST-P(+) foci-specific lipid peroxidation.

13. N. T. P. w. group (2000). "Toxicology and carcinogenesis studies of 2-Butoxyethanol in F344/N rats and B6C3F1 mice (inhalation studies)." National Toxicology Program Technical Report Series **484**(2000).

2-Butoxyethanol is a member of a family of ethylene glycol monoalkyl ethers. It is used extensively as a solvent in surface coatings such as lacquers, enamels, varnishes, and latex paint; in paint thinners, paint stripping formulations, and inks; and in degreasers and industrial and household cleaners. 2-Butoxyethanol was nominated for study because of its widespread use in industrial and consumer applications, the potential for exposure to workers and the general population, and the absence of chronic toxicity data. Male and female F344/N rats and 1360171 mice were exposed to 2-butoxyethanol

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(greater than 99 % pure) by inhalation (primary route of human exposure) for 14 weeks or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, cultured Chinese hamster ovary cells, and the bone marrow of male F344/N rats and B6C3F1 mice. 14-week study in rats. Groups of 10 male and 10 female rats were exposed to 2-butoxyethanol by inhalation at concentrations of 0, 31, 62.5, 125, 250, or 500 ppm, 6 hours per day, 5 days per week for 14 weeks. One female rat in the 250 ppm group was killed moribund during week 8; four females in the 500 ppm group were killed moribund during week 1 and one during week 5. Final mean body weights of females exposed to 500 ppm were significantly less than those of the chamber controls. Clinical findings included abnormal breathing, pallor, red urine stains, nasal and eye discharge, lethargy, and increased salivation and/or lacrimation. Due to vascular thrombosis and infarction in the tail vertebrae of 500 ppm female rats, the tails became necrotic and either sloughed off or were chewed off. The primary effect on the hematopoietic system was an anemia characterized as macrocytic, normochromic, and regenerative in males exposed to 125 ppm or greater and, to a greater extent, in all exposed groups of females. Compared to the chamber controls, kidney weights of males exposed to 500 ppm and females exposed to 125 ppm or greater and liver weights of males exposed to 250 or 500 ppm and females exposed to 125 ppm or greater were significantly increased, and thymus weights of females exposed to 500 ppm were significantly less. In female rats killed moribund, there was considerable histologic evidence of thrombosis in tissues and organs including the nasal cavity, incisors, liver, lung, and heart. In addition to thrombosis, infarction occurred in the vertebrae of the tail resulting in necrosis and loss of the distal portion of the tail. There were also inflammation, necrosis, and ulceration of the forestomach; necrosis and centrilobular degeneration of the liver; renal tubule degeneration; and atrophy of the spleen and thymus. Exposure-related increases in the incidences of Kupffer cell pigmentation, forestomach inflammation and epithelial hyperplasia, bone marrow hyperplasia, splenic hematopoietic cell proliferation, and renal tubule pigmentation were observed in male and/or female rats surviving to the end of the study. 14-week study in mice. Groups of 10 male and 10 female mice were exposed to 2-butoxyethanol by inhalation at concentrations of 0, 31, 62.5, 125, 250, or 500 ppm, 6 hours per day, 5 days per week for 14 weeks. Two male and two female mice exposed to 500 ppm died and two males and two females were killed moribund during the first 2 weeks of the study. Final mean body weights of 125, 250, and 500 ppm male mice were significantly less than those of the chamber controls. Clinical findings were observed only in 500 ppm males and females that died or were killed moribund and included abnormal breathing, red urine stains, and lethargy. Hematologic evaluation indicated an anemia that was characterized as normocytic, normochromic, and regenerative in mice exposed to 62.5 ppm or greater; the anemia was more pronounced in females. Liver weights of males exposed to 500 ppm were significantly greater than the chamber controls. In mice either dying early or killed moribund, there were inflammation, necrosis, and ulceration of the forestomach; mediastinal pleura and peritoneal inflammation associated with the forestomach lesions; liver necrosis; renal tubule degeneration; atrophy of the spleen, thymus, and mandibular and mesenteric lymph nodes; and degeneration of the testis. Exposure-related increases in the incidences of hematopoietic cell proliferation and hemosiderin pigmentation of the spleen, Kupffer cell hemosiderin pigmentation of the liver, inflammation and epithelial hyperplasia of the forestomach, and renal tubule hemosiderin pigmentation occurred in male and/or female mice surviving to the end of

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the study. 2-year study in rats. Groups of 50 male and 50 female rats were exposed to 2-butoxyethanol by inhalation at concentrations of 0, 31.2, 62.5, or 125 ppm, 6 hours per day, 5 days per week for 104 weeks. For hematology and bone marrow analyses, additional groups of 27 male and 27 female rats were exposed to 0, 62.5, or 125 ppm for evaluation at 3, 6, and 12 months and nine male and nine female rats were exposed to 31.2 ppm for evaluation at 3 (hematology only) and 6 months. Survival and Body Weights. Survival of exposed male and female rats was similar to the chamber control groups. The mean body weights of females exposed to 125 ppm were generally less than the chamber control group. Hematology and Bone Marrow Cellularity. The most consistent exposure-related effect on the hematopoietic system was an exposure concentration-related mild macrocytic, normochromic, regenerative anemia present at 3, 6, and 12 months, with females more affected than males. Significant increases in bone marrow cellularity and decreases in the myeloid/ erythroid ratio relative to the chamber controls were observed at all time points in females exposed to 125 ppm, and a decrease in the myeloid/erythroid ratio was observed in males exposed to 125 ppm at 12 months. Pathology Findings. The incidence of benign or malignant pheochromocytoma (combined) of the adrenal medulla in females exposed to 125 ppm was not significantly increased compared to the chamber controls but exceeded the historical control range. Exposure-related increases in the incidences of hyaline degeneration of the olfactory epithelium and Kupffer cell pigmentation of the liver were observed in male and female rats.

2-year study in mice. Groups of 50 male and 50 female mice were exposed to 2-butoxyethanol by inhalation at concentrations of 0, 62.5, 125, or 250 ppm, 6 hours per day, 5 days per week for 104 weeks. For hematology and bone marrow analyses, additional groups of 30 male and 30 female mice were exposed to 0, 62.5, 125, or 250 ppm for evaluation at 3, 6, and 12 months. Survival and Body Weights. Survival of male mice exposed to 125 or 250 ppm was significantly less than that of the chamber control group. The mean body weights of exposed males were generally less than those of the chamber control group during the last 6 months of the study. The mean body weights of exposed female mice were less than those of the chamber control group; the reductions were greater and occurred earlier than those observed in males. Hematology. The most consistent exposure-related effect on the hematopoietic system was an exposure concentration-related minimal normocytic, normochromic, regenerative anemia present at 3, 6, and 12 months, with females affected slightly more than males. Pathology Findings. In females exposed to 250 ppm, incidences of forestomach squamous cell papilloma and squamous cell papilloma or carcinoma (combined) were significantly increased relative to the chamber controls, and these incidences exceeded the ranges in historical chamber controls. In 2-butoxyethanol exposed males, there were possible exposure-related increases in the incidences of squamous cell papilloma of the forestomach, although the increases were not significant and the incidences were within the historical control range for chamber controls. Accompanying these neoplasms in females and, to a lesser extent, in males were exposure-related increases in the incidences of ulcer and epithelial hyperplasia of the forestomach. In male mice exposed to 250 ppm, the incidence of hemangiosarcoma of the liver was significantly increased relative to chamber controls and exceeded the range in historical controls; in addition, there were possible exposure-related increases in the incidence of hepatocellular carcinoma. Incidences of hemosiderin pigmentation in the Kupffer cells were significantly increased in 125 and 250 ppm males and all exposed groups of females. The incidences of splenic hematopoietic cell proliferation and

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hemosiderin pigmentation were generally increased in males and females, and the incidences of bone marrow hyperplasia were increased in males. The incidences of hyaline degeneration of the olfactory and respiratory epithelia of the nose were increased in female mice. Genetic toxicology. 2-Butoxyethanol did not induce mutations in any of the *S. typhimurium* strains tested, with or without induced hamster or rat liver S9. 2-Butoxyethanol induced cycle delay but did not induce either sister chromatid exchanges or chromosomal aberrations in cultured Chinese hamster ovary cells with or without S9. 2-Butoxyethanol did not induce micronuclei in bone marrow cells of male rats or mice administered the chemical by intraperitoneal injection three times at 24-hour intervals. Conclusions. Under the conditions of these 2-year inhalation studies, there was no evidence of carcinogenic activity* of 2-butoxyethanol in male F344/N rats exposed to 31.2, 62.5, or 125 ppm. There was equivocal evidence of carcinogenic activity of 2-butoxyethanol in female F344/N rats based on the increased combined incidences of benign or malignant pheochromocytoma (mainly benign) of the adrenal medulla. There was some evidence of carcinogenic activity of 2-butoxyethanol in male B6C3F1 mice based on increased incidences of hemangiosarcoma of the liver. A marginal increase in the incidences of forestomach squamous cell papilloma and an increase in the incidences of hepatocellular carcinoma may, have been exposure related. There was some evidence of carcinogenic activity of 2-butoxyethanol in female B6C3F1 mice based on increased incidences of forestomach squamous cell papilloma or carcinoma (mainly papilloma). Increased incidences of forestomach neoplasms in male and female mice occurred in groups in which ulceration and hyperplasia were also present. Exposure to 2-butoxyethanol caused a mild regenerative anemia and effects secondary to the anemia.

14. N. T. P. w. group (2001). "Toxicology and carcinogenesis studies of Fumonisin in F344/N rats and B6C3F1 mice (feed studies)." National Toxicology Program Technical Report Series 496(2001).

Fumonisin B1 is a mycotoxin produced by the fungus *Fusarium moniliforme*, one of the major species found in corn. There are no known commercial or medical uses of fumonisin B1. Fumonisin B1 was nominated by the FDA Center for Food Safety and Applied Nutrition for study because of its occurrence in corn and corn-based products in the United States and its toxicity in field exposure of horses and pigs. Male and female F344/N Nctr BR rats and B6C3F1/Nctr BR (C57BL/6N x C3H/HeN MTV-) mice were exposed to fumonisin B1 (92% pure) in feed for 28 days or (greater than 96% pure) for 2 years. 28-day study in rats. Groups of 10 male and 10 female rats were fed diets containing 0, 99, 163, 234, or 484 ppm fumonisin B1 for 28 days. There were no exposure-related deaths in rats. The mean body weights of the 484 ppm groups were significantly less (-16%) than those of the controls. Dietary concentrations of 99, 163, 234, and 484 ppm fumonisin B1 resulted in average daily doses of 12, 20, 28, and 56 mg fumonisin B1/kg body weight for males and females. Additional groups of male and female rats were exposed to the same concentrations of fumonisin B1 for 28 days for clinical pathology studies. The concentrations of creatinine, cholesterol, triglycerides, and total bile acids, as well as activities of the enzymes alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, and gamma-glutamyltransferase, were generally significantly greater in the 484 ppm groups than in the control groups at

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all time points, indicating hyperlipidemia and a hepatic effect. Fumonisin B1 is an inhibitor of ceramide synthase, resulting in an interruption of de novo sphingolipid synthesis. This enzyme inhibition results in increased levels of sphinganine (or increased sphinganine:sphingosine ratio) in tissues and urine. Urinary sphinganine was increased in groups of males exposed to 163 ppm or greater, while urinary sphinganine was increased in all exposed groups of females. The kidney weights, relative to body weight, of all exposed groups of rats were less than those of the control groups, decreasing by approximately 11% in the females and 20% in the males. Apoptosis and degeneration of the kidney were observed in all exposed males and in most females exposed to 163 ppm or greater. The incidences of minimal to mild apoptosis, degeneration, and mitotic alteration of the liver were significantly increased in 234 and 484 ppm males and in females exposed to 163 ppm or greater. The incidences of bile duct hyperplasia were significantly increased in males and females in the 484 ppm groups. In the core study, male rats in all exposed groups and females exposed to 163 ppm or greater had significantly increased percentages of hepatocytes in one or more proliferative (non-G0) states.

28-day study in mice. Groups of 12 male and 12 female mice were fed diets containing 0, 99, 163, 234, or 484 ppm fumonisin B1 for 28 days. There were no exposure-related deaths in mice. The mean body weights of the 484 ppm groups of males were significantly less than those of the controls. Feed consumption by males exposed to 484 ppm was less than that by the controls; dietary concentrations of 99, 163, 234, and 484 ppm fumonisin B1 resulted in average daily doses of approximately 19, 31, 44, and 93 mg/kg for males and 24, 41, 62, and 105 mg/kg for females. Additional groups of male and female mice were exposed to the same concentrations of fumonisin B1 for 28 days for clinical pathology studies. Cholesterol and total bile acid concentrations and alanine aminotransferase and alkaline phosphatase activities were increased at 484 ppm, indicating hyperlipidemia and a hepatic effect. Urinary sphinganine concentrations and sphinganine/sphingosine ratios were increased in 484 ppm male mice. In 484 ppm males and all exposed groups of females, the incidences of hepatocellular necrosis, diffuse periportal hypertrophy, and diffuse centrilobular hyperplasia, as well as hyperplasia of the bile canaliculi and Kupffer cells, were generally significantly greater than those in the controls. Core study males exposed to 99, 163, or 234 ppm had significantly increased incidences of hepatocellular cytoplasmic alteration. Hepatocytes of 484 ppm male mice and all exposed groups of female mice were induced into proliferative (non-G0) states.

2-year study in rats. Groups of 48 male and 48 female rats (40 for 5 ppm groups) were fed diets containing 0, 5, 15, 50, or 150 ppm fumonisin B1 (males) or 0, 5, 15, 50, or 100 ppm fumonisin B1 (females) (equivalent to average daily doses of approximately 0.25, 0.76, 2.5, or 7.5 mg/kg to males and 0.31, 0.91, 3.0, or 6.1 mg/kg to females) for 105 weeks. Additional groups of four male and four female rats were exposed to the same concentrations as the core study animals and were evaluated at 6, 10, 14 or 26 weeks. Survival, Body Weights, and Feed Consumption. Survival, mean body weights, and feed consumption of exposed male and female rats were generally similar to the controls throughout the study. Clinical Pathology Findings. Sphinganine/sphingosine ratios were increased in the urine of 15, 50 and 150 ppm males and 50 and 100 ppm females exposed to fumonisin B1 for up to 26 weeks. The sphinganine/sphingosine ratios were also increased in kidney tissue of 50 and 150 ppm males (85- and 119-fold) and 50 and 100 ppm females (7.8- and 22-fold) at 2 years. Cell Proliferation Analyses. Renal tubule epithelial cell proliferation was increased in 50 and 150 ppm male rats exposed to fumonisin B1 for up to 26 weeks. Renal tubule epithelial

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cell proliferation was marginally increased in 100 ppm females. Organ Weights and Pathology Findings. Kidney weights of 50 and 150 ppm males were less than those of the controls at 6, 10, 14, and 26 weeks and at 2 years. Kidney weights of 100 ppm females were less than those of the controls at 26 weeks, and kidney weights of 15, 50, and 100 ppm females were less than those of the controls at 2 years. At 2 years, there was a significant increase in the incidences of renal tubule adenoma from none in the groups receiving 15 ppm or less to five of 48 in 150 ppm males. Renal tubule carcinomas were not present in male rats receiving 15 ppm or less and occurred in seven of 48 and 10 of 48 male rats in the 50 and 150 ppm groups, respectively. Incidences of apoptosis of the renal tubule epithelium were generally significantly increased in males exposed to 15 ppm or greater for up to 26 weeks. The incidences of focal renal tubule epithelial hyperplasia were significantly increased in 50 and 150 ppm males at 2 years. 2-year study in mice. Groups of 48 male and 48 female mice were fed diets containing 0, 5, 15, 80, or 150 ppm (males) or 0, 5, 15, 50, or 80 ppm (females) fumonisin B1 (equivalent to average daily doses of approximately 0.6, 1.7, 9.7, or 17.1 mg/kg to males or 0.7, 2.1, 7.1, or 12.4 mg/kg to females) for 105 weeks. Additional groups of four male and four female mice were exposed to the same concentrations as the core study animals and were evaluated at 3, 7, 9, or 24 weeks. Survival, Body Weights, and Feed Consumption. Survival of males and females in the 15 ppm groups and of 5 ppm females was significantly greater and survival of 80 ppm males and females was significantly less than that of the control groups. Mean body weights and feed consumption of exposed mice were generally similar to the controls. Organ Weights and Pathology Findings. Liver weights, relative to body weight, were increased 1.3- and 2.9-fold in 50 and 80 ppm females at 2 years. At 2 years, the incidences of hepatocellular adenoma in 50 and 80 ppm females were significantly greater than those in the controls and occurred with a positive trend. Similarly, the incidences of hepatocellular carcinoma increased from none in the groups receiving 0, 5, or 15 ppm fumonisin B1 to 10 of 47 females at 50 ppm and nine of 45 females at 80 ppm. The incidences of hepatocellular hypertrophy were significantly increased in 15, 80, and 150 ppm males and in 50 and 80 ppm females at 2 years. The incidences of hepatocellular apoptosis were significantly increased in 50 and 80 ppm females at 2 years. Conclusions. Under the conditions of these 2-year feed studies, there was clear evidence of carcinogenic activity of fumonisin B1 in male F344/N rats based on the increased incidences of renal tubule neoplasms. There was no evidence of carcinogenic activity of fumonisin B1 in female F344/N rats exposed to 5, 15, 50, or 100 ppm. There was no evidence of carcinogenic activity of fumonisin B1 in male B6C3F₁ mice exposed to 5, 15, 80, or 150 ppm. There was clear evidence of carcinogenic activity of fumonisin B1 in female B6C3F₁ mice based on the increased incidences of hepatocellular neoplasms. The sphinganine/sphingosine ratios were increased in the urine and the kidney tissue of rats receiving diets containing fumonisin B1. There was evidence of apoptosis and increased cell proliferation of the renal tubule epithelium in exposed rats, particularly in those groups of males that developed renal tubule neoplasms. Increased incidences of hyperplasia of the renal tubule epithelium also occurred in these groups of male rats. In mice exposed to the higher concentrations of fumonisin B1, males and females had increased incidences of hepatocellular hypertrophy and females had increased incidences of hepatocellular apoptosis.

15. Nyska, A., et al. (2004). "Association of liver hemangiosarcoma and secondary iron overload in B6C3F1 mice--the National Toxicology Program experience." Toxicol Pathol. **32**(2): 222-228. [Toxicologic pathology].

The literature evidencing the role of iron in promoting a range of neoplasms in humans and animals prompted us to search for a possible association between chemically induced hemosiderosis and hemangiosarcomas in the liver of mice in selected studies conducted by the National Toxicology Program (NTP). Its historical control database was examined for studies in which treatment-related liver hemangiosarcoma was noted; 130 consecutive NTP studies in B6C3F1 mice from Technical Report (TR)-340 to TR-493 were evaluated. Three compounds (2-butoxyethanol, p-nitroaniline, and para-chloroaniline) were associated with a relatively high incidence of Kupffer cell pigmentation consisting of hemosiderin in both sexes; only the male mice developed a relatively low incidence of treatment-related hemangiosarcoma. With a fourth compound (o-nitroanisole), a relatively low incidence (16/50, high-dose males) of chemical-related hemosiderosis was noted, with no associated increase of hemangiosarcoma. Two chemicals (pentachlorophenol and tetrafluoroethylene) increased the incidence of liver hemangiosarcoma in male and female mice, with no increase in Kupffer cell pigmentation. The overall association between liver hemangiosarcoma and Kupffer cell pigmentation was highly significant ($p < 0.001$). The cause for hemosiderosis in all cases was the erythrocytic hemolytic effect of the compounds. The reason for the sex-increased susceptibility for development of hemangiosarcoma is unknown but may be due to a hormone-related, reduced antioxidative defensive capacity through modulation of the activities of antioxidative enzymes.

16. Olivieri, N. F., et al. (2011). A pilot study of subcutaneous decitabine in β -thalassemia intermedia. Blood. **118**: 2708-2711. [Blood].

Ineffective erythropoiesis, the hallmark of β -thalassemia, is a result of α -non- α ; globin chain imbalance. One strategy to redress globin-chain imbalance is to induce γ -globin gene (HBG) expression. Repression of HBG in adult erythroid cells involves DNA methylation and other epigenetic changes. Therefore, the cytosine analog decitabine, which can deplete DNA methyltransferase 1 (DNMT1), can potentially activate HBG. In 5 patients with β -thalassemia intermedia, a dose and schedule of decitabine intended to deplete DNMT1 without causing significant cytotoxicity (0.2 mg/kg subcutaneous 2 times per week for 12 weeks) increased total hemoglobin from 7.88 \pm 0.88 g/dL to 9.04 \pm 0.77 g/dL ($P = .004$) and absolute fetal hemoglobin from 3.64 \pm 1.13 g/dL to 4.29 \pm 1.13 g/dL ($P = .003$). Significant favorable changes also occurred in indices of hemolysis and red blood cell densitometry. Consistent with a noncytotoxic, differentiation altering mechanism of action, the major side effect was an asymptomatic increase in platelet counts without erythrocyte micronucleus or VDJ recombination assay evidence of genotoxicity. This study was registered at www.clinicaltrials.gov as #NCT00661726.

17. Shahbazi, M. A., et al. (2013). "The mechanisms of surface chemistry effects of mesoporous silicon nanoparticles on immunotoxicity and biocompatibility." Biomaterials. **34**(31): 7776-7789. [Biomaterials].

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Despite steadily increasing insights on the biocompatibility of PSi nanoparticles (NPs), an extensive biosafety study on the immune and red blood cells (RBCs) is still lacking. Herein, we evaluated the impact of the PSi NPs' surface chemistry on immune cells and human RBCs both *in vitro* and *in vivo*. Negatively charged hydrophilic and hydrophobic PSi NPs caused less ATP depletion and genotoxicity than the positively charged amine modified hydrophilic PSi NPs, demonstrating the main role of PSi NPs' surface charge on the immunocompatibility profile. Furthermore, cells with lower metabolic activity, longer doubling time, and shorter half-life were more sensitive to the concentration- and time-dependent toxicity in the following order: T-cells > monocytes > macrophages > B-cells. RBC hemolysis and imaging assay revealed a significant correlation between the surface chemistry, the amount of the PSi NPs adsorbed on the cell surface and the extent of morphological changes. The *in vivo* results showed that despite mild renal steatosis, glomerular degeneration, hepatic central vein dilation and white pulp shrinkage in spleen, no notable changes were observed in the serum level of biochemical and hematological factors. This study is a comprehensive demonstration of the mechanistic direct and indirect genotoxicity effects of PSi NPs, elucidating the most influencing properties for the PSi NPs' design.

18. Whysner, J. and C. X. Wang (2001). Hepatocellular iron accumulation and increased cell proliferation in polychlorinated biphenyl-exposed Sprague-Dawley rats and the development of hepatocarcinogenesis. *Toxicol Sci.* **62**: 36-45. [Toxicological sciences : an official journal of the Society of Toxicology].

Polychlorinated biphenyls (PCBs) are liver-tumor promoters in rodents, but the underlying mechanisms have not been fully elucidated. Tissue sections from the PCB bioassay reported by Mayes et al. 1998, *Toxicol Sci.*, 41-66, were evaluated by histopathological techniques that included immunohistochemistry. In females, and to a much lesser extent in males, iron accumulation in hepatocytes was found at the 26th-week sacrifice, which was pronounced in the mid- and high-dose Aroclor-1254 and -1260 groups. At 52 weeks, large accumulations of iron were also present in Kupffer cells of females, and dose-related increases in proliferating cell nuclear antigen (PCNA) hepatocyte labeling indices were found in both males and females. These changes preceded the formation of liver tumors, which were not generally found until 78 weeks. Glutathione S-transferase placental (GSTP) positive foci were present at 52 weeks in high-dose Aroclor-1254 and -1260 female groups, and small foci were found in some Aroclor 1254-exposed female rats at 26 weeks, along with centrilobular hepatocytes expressing GSTP. The results of this study suggest that PCB-induced iron accumulation in hepatocytes is an early event that may be related to tumor formation, especially in female rats. In both males and females, increases in cell proliferation at 52 weeks were statistically significantly correlated with tumor incidences at termination among the various PCB dosage groups. Consequently, iron accumulations producing oxidative damage, and enhanced cell proliferation resulting in tumor promotion may be components in the mode of action for PCB-induced hepatocarcinogenesis in rodents.

19. Wu, W., et al. (2010). Biocompatibility of Fe₃O₄/DNR magnetic nanoparticles in the treatment of hematologic malignancies. Int J Nanomedicine. 2010; 5:1079-84. [International journal of nanomedicine].

PURPOSE: The objectives of this research were to assess the biocompatibility of self-assembled Fe₃O₄ magnetic nanoparticles (MNPs) loaded with daunorubicin (DNR), ie, (Fe₃O₄)-MNPs/DNR, and to explore their potential application in the treatment of hematologic malignancies.

METHODS: A hemolysis test was carried out to estimate the hematologic toxicity of Fe₃O₄-MNPs/DNR and a micronucleus assay was undertaken to identify its genotoxicity. Fe₃O₄-MNPs/ DNR were injected intraperitoneally into mice to calculate the median lethal dose (LD₅₀). The general condition of the mice was recorded, along with testing for acute toxicity to the liver and kidneys.

RESULTS: Hemolysis rates were 2.908%, 2.530%, and 2.415% after treatment with different concentrations of Fe₃O₄-MNPs/DNR. In the micronucleus assay, there was no significant difference in micronucleus formation rate between the experimental Fe₃O₄-MNPs/DNR groups and negative controls (P > 0.05), but there was a significant difference between the experimental groups and the positive controls (P < 0.05). The LD₅₀ of the Fe₃O₄-MNPs/DNR was 1009.71 mg/kg and the 95% confidence interval (CI) was 769.11-1262.40 mg/kg, while that of the DNR groups was 8.51 mg/kg (95% CI: 6.48-10.37 mg/kg), suggesting that these nanoparticles have a wide safety margin. Acute toxicity testing showed no significant difference in body weight between the treatment groups at 24, 48, and 72 hours after intraperitoneal injection. The mice were all in good condition, with normal consumption of water and food, and their stools were formed and yellowish-brown. Interestingly, no toxic reactions, including instability of gait, convulsion, paralysis, and respiratory depression, were observed. Furthermore, alanine transaminase, blood urea nitrogen, and creatinine clearance in the experimental Fe₃O₄-MNPs/ DNR groups were 66.0 ± 28.55 U/L, 9.06 ± 1.05 mmol/L, and 18.03 ± 1.84 μmol/L, respectively, which was not significantly different compared with the control and isodose DNR groups.

CONCLUSION: Self-assembled Fe₃O₄-MNPs/DNR appear to be highly biocompatible and safe nanoparticles, and may be suitable for further application in the treatment of hematologic malignancies.

20. Zhang, W. F., et al. (2009). Biocompatibility study of theophylline/chitosan/beta-cyclodextrin microspheres as pulmonary delivery carriers. J Mater Sci Mater Med. 20: 1321-1330. [Journal of materials science. Materials in medicine].

To evaluate the biocompatibility of the theophylline/chitosan/beta-cyclodextrin microspheres, which has a potential application in pulmonary delivery system. The detection of LDH and protein in BALF was examined acute cell toxicity, hemolysis test was carried out to estimate blood toxicity; Micronucleus Test was reckoned to identify genotoxicity, MTT assay was used to evaluate in vitro cytotoxicity, and muscle implantation investigated the tissue biocompatibility. The results demonstrated that the

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total contents of protein and LDH in BALF were not significantly different from that of normal group. The experiments showed that the cytotoxicity was depended on the concentration and had no cytotoxicity at low concentration and no hemolysis activity. The micronucleus frequency of MS B was 0.99 per thousand, which showed no genotoxic effects either. The results of implantation showed that the microspheres had no effect on hemoglobin and no toxicity in the liver and kidney. The inflammations of muscle tissue were not significantly different from that of operative suture, therefore, the MS B possess high good biocompatibility and can be applied in pulmonary sustained release systems.

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1. Udden, M. M. and C. S. Patton (2005). "Butoxyacetic acid-induced hemolysis of rat red blood cells: effect of external osmolarity and cations." Toxicol Lett **156**(1): 81-93.

Hemolysis is the principal toxicity of acute exposure to ethylene glycol monobutyl ether (EGBE) in rats. EGBE itself is not an active hemolytic agent, but its metabolite, butoxyacetic acid (BAA) formed as a result of dehydrogenase activity is a potent hemolysin. Here we address the role of osmolarity and cation composition of the suspending buffers in the mechanism of BAA-induced hemolysis of rat red blood cells in vitro. Rat erythrocytes were protected from BAA-induced cell swelling and hemolysis by the addition of sucrose to the suspending media. Hemolysis and cell swelling were also reduced by replacing external sodium with potassium. When calcium was not present in the suspending medium or when chelated by EGTA, hemolysis was increased after 2 h incubation with 1 mM or 2 mM BAA. Addition of as little as 0.05 mM CaCl₂ reduced hemolysis significantly while the addition of MgCl₂ had no effect. The dose-response relationship between BAA concentration and hemolysis determined in the presence or absence of calcium showed an increased effect of BAA in the absence of calcium. BAA-induced spherocytosis and cell fragmentation were more pronounced in the absence of calcium. The time course of BAA-induced hemolysis in the presence and absence of calcium demonstrated that the effect of calcium is to delay the onset of hemolysis. Increased intracellular calcium as a result of exposure to BAA was verified by atomic absorption spectroscopy. Charybdotoxin, an inhibitor of the calcium activated potassium channel, blocked the protective effect of calcium suggesting that the delay of onset of hemolysis in the presence of calcium is due to potassium loss caused by this channel. We conclude that the mode of action of BAA is to cause a colloid osmotic lysis of the rat red blood cell. Hemolysis requires external sodium and is associated with calcium uptake. Calcium appears to delay the onset of hemolysis. We speculate that: (1) BAA causes sodium and calcium to enter the cell; (2) calcium initially has a protective

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effect via the calcium activated potassium channel which facilitates the loss of potassium thereby, compensating for the osmotic effect of increased cell sodium; (3) calcium subsequently may have other deleterious effects through activation of proteases and externalization of phosphatidylserine in the exterior leaflet of the membrane.