

This document was developed by NIEHS/NTP staff to facilitate internal and external review of a proposed research program prior to designing and conducting toxicology studies. The purpose of the research concept document is to outline the general elements of a research program that would address the specific public health concerns that prompted the nomination of the substance or issue for study. It may also encompass substance-specific studies that address larger public health issues or topics in toxicology. Additional information about the nomination, review, and selection of substances for study by the NTP is provided at *Nominations to the NTP Testing Program* (<http://ntp.niehs.nih.gov/go/nom>). A draft version of this research concept was reviewed by the NTP Board of Scientific Counselors at a public meeting on December 6, 2007 (<http://ntp.niehs.nih.gov/go/9741>) and subsequently approved by the NTP Executive Committee.

NTP Research Concept: 2',2'''-Dithiobisbenzanilide

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Nomination Background and Rationale

2',2'''-Dithiobisbenzanilide (DTBBA) was nominated by the National Cancer Institute (NCI) for genotoxicity and metabolism studies based on the lack of these data and suspicion of toxicity based on the structure of the chemical (<http://ntp.niehs.nih.gov/go/29287>).

DTBBA is a high production chemical with potential for occupational and consumer exposure. The major use for DTBBA is as a peptizing agent (plasticizer) for natural and synthetic rubber. Peptizers reduce viscosity of rubber, thus allowing the material to be shaped and molded into final end products. DTBBA is used in the manufacture of tires and other solid rubber goods, e.g. belts, cables, hoses, as well as in the rubber components of diverse equipment and products used commercially and by consumers. Rubber industry workers are potentially exposed to DTBBA by the inhalation and dermal routes during the compounding and mixing process. The general population may be exposed to small amounts of DTBBA that leach from consumer products, such as rubber and latex gloves, and/or are released into the environment during the manufacture or disposal of DTBBA-containing products. DTBBA is approved by the FDA as a plasticizer for use in materials that may come into contact with food during manufacture, processing, and packaging; and DTBBA has been cited in some U.S. patents for use in pharmaceutical formulations.

A structurally similar chemical, 2,2'-dithiobis-4'-(sulfamoyl)benzanilide, has been shown to have antiretroviral activity in a lymphocyte-derived cell line. No standards or guidelines have been set by NIOSH or OSHA for exposure levels of DTBBA in the workplace, nor is DTBBA currently regulated as a hazardous substance in the U.S. DTBBA has been classified as an environmentally hazardous substance and as an aquatic toxicant in Europe, based on detection of the chemical in wastewater discharged from rubber processing sites and acute toxicity data from aquatic organisms, including fish. The acute toxicity in mammals appears to be low. The reported oral LD₅₀ of DTBBA in rats was > 4 g/kg and the dermal LD₅₀ was 10 g/kg in rabbits. There are no subchronic or carcinogenicity studies in animals or epidemiological studies of DTBBA in humans reported in the literature. Increased incidence of cancer and contact dermatitis has been reported among workers in the rubber industry; however, health evaluations generally are unable to identify the specific causative agent(s). DTBBA is a potential skin and respiratory irritant and may produce skin sensitization. The chemical was found to be a medium-strong sensitizer when applied in pure form to the skin of male guinea pigs. There are no reported studies for mutagenicity or metabolism of DTBBA.

Key Issues

The extent of exposure to humans and the toxic potential of DTBBA have not been adequately determined, although production of the chemical has increased substantially within the past few years (≤ 0.5 million to >1 million lbs/year from 1994 to 2002). Therefore, the NCI has identified the need for a complete toxicological characterization of DTBBA, including subchronic studies in animals, mutagenicity studies, identifying *in vivo* metabolites, and determining the levels of DTBBA and its degradation products in the environment. The latter information is essential for determining the relevance of exposure to DTBBA and its degradation products to the general population, but is beyond the scope of work conducted by the National Toxicology Program (NTP). A key issue identified for initial investigation by the NTP is to determine the potential reactivity of DTBBA. Sensitization studies in guinea pigs indicate that DTBBA is reactive in skin and may undergo metabolism in the tissue. However, these results do not establish the extent of dermal absorption or systemic exposure. Further, the mutagenic potential of DTBBA or its potential for metabolism to mutagenic or reactive metabolites is unknown. It has been speculated that DTBBA may undergo metabolism to benzanilide or benzamide derivatives possessing biological activity.

Proposed Research Program

The main goals of this research project are to investigate the *in vivo* fate of DTBBA and to evaluate the mutagenicity of DTBBA in a standard battery of genotoxicity tests. These studies are considered high priority for toxicological characterization of the chemical. The specific aims of the research project are to:

1. Determine the *in vitro* mutagenic potential of DTBBA. Data from these studies may also be useful in characterizing the potential for DTBBA to form reactive metabolites.
2. Determine the potential for absorption of DTBBA through human and/or rodent skin, *in vitro*.
3. Investigate *in vitro* metabolism of DTBBA in human and/or rodent liver preparations.
4. Determine bioavailability and characterize metabolism of DTBBA in rodents by the oral and dermal routes.

Significance and Expected Outcome

DTBBA is the preferred peptizing agent for use in natural and synthetic rubber and has recently become a high production chemical. Consequently, the potential for human exposure has increased; however, the toxicity of DTBBA has not been adequately characterized. The proposed research project would determine the potential for mutagenicity, absorption, and metabolic activation of DTBBA. Results from this work would provide data critical for determining the potential toxicity of DTBBA and would be used to determine the need for subchronic and long-term toxicity studies in rodents. Ultimately, these studies would provide data used to characterize risks associated with exposure to DTBBA and to determine safe exposure limits for workers and consumers.

References and Supporting Materials

Domagala, J.M. *et al.* (1997) A new class of anti-HIV-1 agents targeted toward the nucleocapsid protein NCp7: The 2,2'-dithiobisbenzamides. *Bioorganic and Medicinal Chemistry*, **5**, 569-579.

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