Captafol/*o*-Nitrotoluene Expert Panel Report

Part B – Recommendation for listing status for o-Nitrotoluene in the RoC and Scientific Justification for the Recommendation

The Report on Carcinogens (RoC) expert panel for Captafol/*ortho*-Nitrotoluene met at the Sheraton Chapel Hill Hotel on October 15 & 16 2007, to peer review the draft background document on captafol and make a recommendation for its listing status in the 12th Edition of the RoC. Members of the expert panel are as follows:

Lauren Zeise, Ph.D. (Chair)
California Environmental Protection Agency
Office of Environmental Health Hazard Assessment

Michael Elwell, D.V.M., Ph.D.
Covance Laboratories, Inc.
Department of Pathology

Penelope A. Fenner-Crisp, Ph.D., D.A.B.T.
Independent Consultant
(Retired from the International Life Science Institute and the U.S. Environmental Protection Agency)

Gregory L. Kedderis, Ph.D.
Independent Consultant

Steven Markowitz, M.D.
Queens College, City University of New York
Center for the Biology of Natural Systems

Robert C. Millikan, D.V.M., Ph.D.
University of North Carolina,
School of Public Health,
Department of Epidemiology

Shane S. Que Hee, Ph.D.
University of California, Los Angeles
School of Public Health, Department of Environmental Health Sciences

Thomas J. Slaga, Ph.D.
University of Texas Health Science Center
Department of Pharmacology

Alexander W. Teass, Ph.D.
(Retired from the National Institute of Occupational Safety & Health)

The recommendation follows this page.

Part B - Recommendation for listing status for *o*-Nitrotoluene in the RoC and the scientific justification for the recommendation

Overall Evaluation

Following a discussion of the body of knowledge, including the strengths and weaknesses for each section of the background document, the expert panel applied the RoC listing criteria and made a recommendation for the listing status of *o*-nitrotoluene in the RoC. The expert panel recommended by a vote of 7 yes/0 no, 1 absent, that *o*-nitrotoluene should be listed in the RoC as *reasonably anticipated to be a human carcinogen* based on sufficient evidence of carcinogenicity in experimental animals and supporting mechanistic evidence. The expert panel also voted (7 yes/0 no, 1 absent) that there is evidence that a significant number of people in the United States are or have been exposed to *o*-nitrotoluene.

The major considerations discussed that led to this recommendation include:

Human Exposure

The opportunity for human exposure to *o*-nitrotoluene is significant. In the United States, *o*-nitrotoluene is produced by one company and is available from 11 suppliers. *o*-Nitrotoluene is used for the production of dyes (magenta and various azo and sulfur dyes), explosives, and organic chemicals used in the petrochemical, pesticide, pharmaceutical, and rubber industries. As a high-production-volume chemical, its annual U.S. production was between 10 million and 50 million pounds for each four-year reporting period from 1986 to 2002.

o-Nitrotoluene is an environmental contaminant in the United States. It has been found in ambient workplace air at levels as high as 47 ng/m^3; in liquid effluent from plants manufacturing 2,4,6-trinitrotoluene and 2,4-dinitrotoluene up to 16 mg/L; in surface water near former munitions plants up to 0.022 mg/L; and in groundwater at facilities for production of munitions at levels as high as 140 mg/L and for military training facilities at levels as high as 0.025 mg/L. Evidence suggests *o*-nitrotoluene is not persistent in air or surface water and does not bioaccumulate appreciably in aquatic organisms. However, it is formed by anaerobic reduction of trinitrotoluene and is a contaminant of concern at military munitions sites.

Human Cancer Studies

The available epidemiologic evidence is insufficient to establish the carcinogenicity of *o*-nitrotoluene in humans. There is only one epidemiologic study that directly addressed *o*-nitrotoluene exposure. In the subgroup of dye workers who were not exposed to benzidine and naphthylamines, there was a very high relative risk of bladder cancer, though this subgroup was also exposed to *o*-toluidine and 4,4'-methylene bis(2-methylaniline). There are two other studies that found excess bladder cancer among workers exposed to magenta dye, but these studies do not specifically address exposure to *o*-nitrotoluene. Thus, because the isolated effect of *o*-nitrotoluene cannot

be determined from available studies, the human evidence is insufficient to establish its human carcinogenicity.

Three epidemiologic studies of exposure to dinitrotoluenes are available and collectively suggest elevations in hepatobiliary, urothelial, renal, and possibly colon cancer. Two studies that involved *o*-toluidine exposure reported excess bladder cancer incidence. The relevance of these studies for carcinogenicity of o-nitrotoluene is uncertain, because they are analogues of *o*-nitrotoluene, or, in the case of o-toluidine, a minor metabolite of *o*-nitrotoluene. In addition, with the exception of the Ward et al study (1991), these studies involve simultaneous exposure to multiple compounds. Thus, available epidemiologic evidence is insufficient to identify the specific effect of *o*-nitrotoluene on human cancer.

Studies in Experimental Animals

There is sufficient evidence of carcinogenicity in experimental animals. Long-term feeding studies of *o*-nitrotoluene were conducted in both sexes of mice and rats. These studies were well-designed, conducted and adequate for evaluating the carcinogenicity of *o*-nitrotoluene. In a two-year carcinogenicity study in mice, *o*-nitrotoluene caused tumors of the large intestine (carcinoma), liver (adenoma or carcinoma; in females), and circulatory system (hemangiosarcoma). In male rats, mesothelioma of the epididymis was first observed in a 13-week toxicity study of *o*-nitrotoluene. Mesothelioma of epididymis and testis as well as cholangiocarcinoma of the liver were also increased in a subsequent 26-week and stop-exposure studies in male rats. This early onset is an unusual finding. In this study preneoplastic glutathione *S*-transferase placental form positive foci (GST-P+) of the liver were also increased in dosed animals. In the 2-year carcinogenicity study in rats, *o*-nitrotoluene caused tumors of the mammary gland (fibromadenoma), skin (subcutaneous lipoma [males], and fibroma or fibrosarcoma), liver (hepatocellular carcinoma [males] and adenoma), lung (alveolar/bronchiolar adenoma or carcinoma in males), and mesothelium (malignant mesothelioma) of the epididymis and testis.

Other Relevant Data

o-Nitrotoluene has been shown to be absorbed after oral administration to rats and mice; the available evidence indicates that absorption occurs in humans. The pathways of metabolism identified in rodents are expected to occur in humans as well.

Significantly increased incidences of tumors in rats or mice, including mammary gland, skin, lung, large intestine, and hemangiosarcomas support the concept that activation pathways in addition to the one related to the liver tumors may exist.

The results of the genotoxicity studies are mixed. *o*-Nitrotoluene did not cause mutations in prokaryotic systems. In mammalian *in vitro* systems, it induced (1) sister chromatid exchange in Chinese hamster ovary (CHO) cells; (2) chromosomal aberrations in Chinese hamster lung cells (CHL) and human peripheral lymphocytes (but not in CHO cells); and (3) micronuclei in CHL cell line. It did not induce DNA repair

in rat or human hepatocytes. The lack of genotoxic effects of *o*-nitrotoluene in some *in vitro* studies is consistent with the need for metabolism by both mammalian and bacterial enzymes. In rats exposed to *o*-nitrotoluene *in vivo*, DNA adducts and increased DNA repair were detected in males but not females. *o*-Nitrotoluene induced a slight increase in normochromatic micronuclei in high-dose male mice (equivocal response) but did not induce micronuclei in the bone marrow of male rats (polychromatic), male mice (polychromatic), or female mice (normochromatic). The genotoxicity of the *o*-nitrotoluene, as measured by the *in vivo-in vitro* DNA repair assay in rats, depends on metabolism (both mammalian and bacterial). DNA repair was induced only in male rats with an intact intestinal microflora. Incubation of *o*-nitrotoluene *in vitro* with hepatocytes isolated from male rats failed to induce DNA repair.

Report Approved _s/Lauren Zeise_____Date __1/11/08____
Lauren Zeise, Ph.D. (Chair)

Proper Signatures
Treat as signed, § 1.4(d)(2)