NTP Research Concept: 2-Methoxy-4-nitroaniline (4-nitro-o-anisidine)

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Nomination Background and Rationale
2-Methoxy-4-nitroaniline was nominated in 2006 by the National Cancer Institute for complete toxicological characterization and evaluation of carcinogenic potential (http://ntp.niehs.nih.gov/go/29287). The basis for the nomination was high and increasing production volume (which most recently ranged from >500,000 to 1,000,000 pounds based on the U.S. EPA's 2002 Inventory Update Rule), the potential for worker exposures, the lack of adequate toxicology and carcinogenicity data, positive mutagenicity data, and the finding that a closely related chemical, 2-methoxy-5-nitroaniline, was a multisite carcinogen when tested by the NTP.

2-Methoxy-4-nitroaniline is used in dyeing processes in the textile industry (e.g., dyeing cotton), as a chromogenic agent in printing processes, and as an intermediate in the synthesis of azo dyes and Pigment Yellow 74, a high production volume chemical which has applications in yellow tattoo inks, emulsion paints, toy enamels, printing inks, and traffic paints. It is also a constituent of pigment pastes for paper and adhesives. The most likely source of human exposure occurs during the production, use, and disposal of 2-methoxy-4-nitroaniline containing products. Occupational exposure to dye dust during processing and handling poses the greatest risk of exposure. Inhalation or dermal exposure to dye dust can lead to asthma, eczema, and allergic responses. There are a total of 35 synonyms and trade names listed for 2-methoxy-4-nitroaniline.

There is little information in the peer reviewed literature on the toxicology of 2-methoxy-4-nitroaniline. It is not a dermal irritant or sensitizer. In a data summary it was reported that following i.p. injection two major metabolites were identified in the urine of male rats: 2-methoxy-p-phenylenediamine (nitroreduction) and 2-amino-5-nitrophenol (o-demethylation) and their N-acetylated derivatives. A summary report of a 28-day oral toxicology study indicated that it causes myocardial necrosis, which is consistent with reports in the literature that 2-methoxy-p-phenylenediamine as well as a number of other substituted phenylenediamines, cause necrosis of both skeletal and cardiac muscle in rodents.

2-Methoxy-4-nitroaniline has been consistently positive in bacterial mutagenicity assays in the presence of activation with mixed results in the absence of activation. The lyophilized urine of rats administered 2-methoxy-4-nitroaniline was also reported to be mutagenic in bacteria, consistent with the reported bacterial mutagenicity of 2-methoxy-p-phenylenediamine.

2-Amino-5-nitrophenol (a potential metabolite of 2-methoxy-4-nitroaniline as noted above) has been evaluated for carcinogenic potential by the NTP. It was mutagenic with and without exogenous metabolic activation, induced forward mutations in mouse lymphoma cells in the absence of metabolic activation, and an increase in chromosomal aberrations and sister chromatid exchanges in CHO cells both in the presence and absence of exogenous metabolic activation. 2-Amino-5-nitrophenol administered by gavage produced some evidence of

carcinogenic activity in male F344 rats based on increased incidences of acinar cell adenomas of the pancreas.

NTP has also examined the carcinogenic potential of several structurally-related compounds. 2-Methoxy-5-nitroaniline administered in feed significantly increased the incidence of a variety of skin tumors in male and female rats and hepatocellular carcinomas in female mice. 2-Amino-4-nitrophenol, a potential metabolite of 2-methoxy-5-nitroaniline, was mutagenic in salmonella in the presence of metabolic activation and in mouse lymphoma cells in the absence of activation. In 2-year studies 2-amino-4-nitrophenol administered by gavage increased the incidence of renal tubular hyperplasia and renal cortical adenomas in male rats but was not carcinogenic in mice.

Key Issues

A key issue will be what route or routes of exposure to use for the proposed studies. Exposure to 2-methoxy-4-nitroaniline occurs in occupational settings and involves inhalation and dermal exposure to the dry powdered material. A complete toxicological characterization would require prechronic studies by both routes.

Another key issue is the extent to which 2-methoxy-4-nitroaniline or its metabolites are DNA reactive. The available data indicate that it is a bacterial mutagen in the presence of activation but the results are unclear in the absence of activation. If 2-methoxy-5-nitroaniline or a metabolite forms DNA adducts, this information combined with the known carcinogenicity of structurally related chemicals may preclude the need for a 2-year study to evaluate the carcinogenic potential of 2-methoxy-4-nitroaniline.

Proposed Research Program

The overall goal of this research is to characterize the toxicology and carcinogenic potential of 2-methoxy-4-nitroaniline. The specific aims of the proposed studies are to:

- Determine absorption by dermal and oral routes
- Identify major metabolites
- Conduct subchronic toxicity studies by appropriate routes with special attention to heart and skeletal muscle as potential target organs
- Conduct subchronic toxicity studies beginning with *in utero* exposure
- Evaluate reproductive toxicity
- Evaluate the mutagenicity and DNA reactivity of parent and metabolites
- If necessary, conduct a 2-year study to evaluate chronic toxicity and carcinogenic potential

Significance and Expected Outcome

The increasing production and use of 2-methoxy-4-nitroaniline in the dyeing and printing industries increases the risk of occupational exposure, however the information available for this compound is inadequate to determine the risk associated with this exposure. The 1981-1983 National Occupational Exposure Survey estimated that approximately 54,867 workers (11,681 females) were exposed to Pigment Yellow 74; workers in the textile, printing and publishing, and the wholesale trade (durable goods and special trade contractors) industries had the highest potential exposure. No epidemiological or case studies of cancer risk in humans were reported however 2-methoxy-4-nitroaniline is released when sunlight interacts with yellow tattoo

pigments, and there are over 90 studies reporting an association between tattoos and skin cancer. Although consumer exposure has not been demonstrated, the general public may be exposed in textile materials, printing inks, and/or dyes that can leach from products containing Pigment Yellow 74 (for example: washing dyed clothes). Several substituted phenylenediamines are myotoxic in rodents, 2-methoxy-4-nitroaniline is a bacterial mutagen, and several structurally similar compounds are mutagens and carcinogens. The data generated by the proposed research would completely characterize the toxicity of 2-methoxy-4-nitroaniline and evaluate its carcinogenic potential by a relevant route of exposure.