Research Concept: 2-Methoxy-4-nitroaniline

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NTP Board of Scientific Counselors

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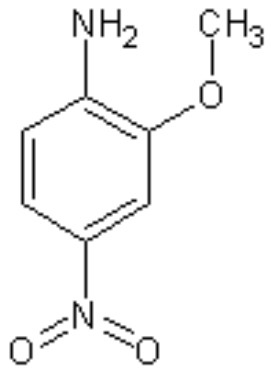
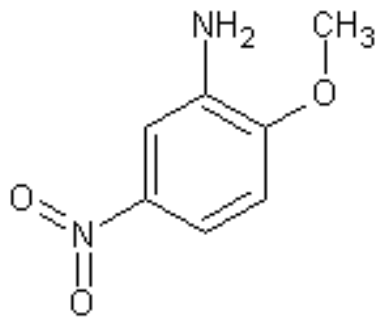
Nomination

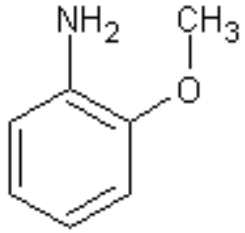


- Nominated by NCI in 2006
- Increased production >500,000-1,000,000 lbs.
- Carcinogenic potential unknown
- Inadequate characterization of toxicity
- Significant potential for occupational exposure
- Consumer exposure undocumented, however 2-methoxy-4-nitroaniline is used in the synthesis of pigment yellow 74 which is present in numerous consumer products, yellow tattoo inks, and printing inks

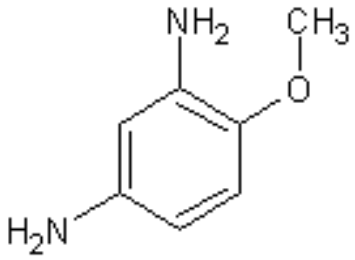
Structure-activity



2-methoxy-5-nitroaniline



o-anisidine



2,4-diaminoanisole

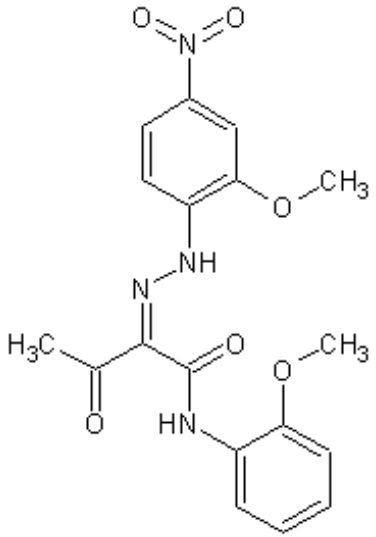
- 2-methoxy-5-nitroaniline: skin neoplasms in rats and hepatocellular neoplasms in mice
- o-anisidine: transitional cell carcinomas of the bladder in rats and mice
- 2,4-diaminoanisole: skin and thyroid neoplasms in rats and thyroid neoplasms in mice

Human exposure

- Primarily occupational exposure associated with handling dry powder during dye manufacture
- No epidemiology studies or case reports dealing specifically with exposure to 2-methoxy-4-nitroaniline
- NOES estimates that 54,867 workers exposed to pigment yellow 74 of which 11,681 were female
- Workers in the apparel, textile, and printing industries at highest risk for exposure to pigment yellow 74
- No standards or guidelines set by NIOSH or OSHA for occupational exposure to or workplace allowable levels of 2-methoxy-4-nitroaniline
- 2-Methoxy-4-nitroaniline is not on the ACGIH list of compounds for which recommendations for a TLV are made.

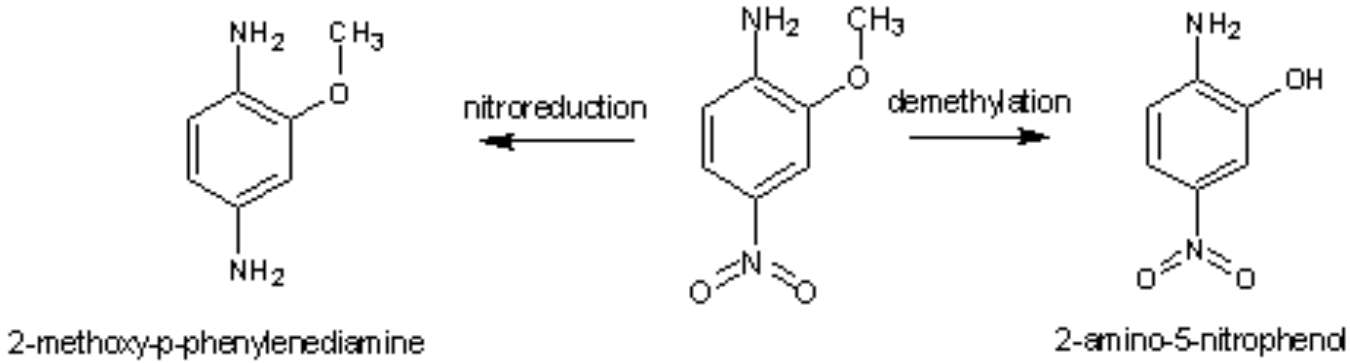
Human exposure

- Pigment yellow 74 is used in yellow tattoo inks, printing inks, fabric dyeing
- there are over 90 studies reporting an association between tattoos and skin cancer
- 2-methoxy-4-nitroaniline is not released during the microsomal metabolism of PY74



Background

- Little information in peer reviewed literature
- Preliminary report indicates major metabolites are 2-methoxy-p-phenylenediamine (nitroreduction) and 2-amino-5-nitrophenol (o-demethylation)



- 2-methoxy-4-nitroaniline selectively induces CYP1A2 in rat liver but not in the livers of other rodents

Background

- Preliminary reports indicate toxicity to skeletal muscle and heart
- p-Phenylenediamine (1,4-diaminobenzene) is myotoxic in humans causing extensive rhabdomyolysis and consequent renal failure
- p-Phenylenediamine and several N-methylated p-phenylene-diamines are myotoxic in rats causing necrosis of skeletal and cardiac muscle
- 2-methoxy-p-phenylenediamine, a metabolite of 2-methoxy-4-nitoaniline, causes necrosis of skeletal muscle (gastrocnemius, diaphragm, tongue) in rats at doses of 8.4 mg/kg or greater
- 2-methoxy-p-phenylenediamine was more myotoxic than p-phenylenediamine which required a dose of 36 mg/kg to produce myotoxicity in rats

Key Issue: Route(s) of exposures

- occupational exposure is primarily by dermal and/or inhalation routes
- Both skin and respiratory tissue have metabolic capability for metabolizing 2-methoxy-4-nitroaniline
- 2-methoxy-5-nitroaniline and 2,4-diaminoanisole are dermal carcinogens when administered in feed*
- No information on dermal absorption
- All other compounds in this structure class have been evaluated for carcinogenic potential using oral exposure

Specific Aim 1: conduct ADME

- Conduct ADME studies by oral, dermal, and inhalation routes of exposure including identification of major metabolites
- If there is significant bioavailability (blood concentration) of parent compound following dermal or inhalation exposure, then oral administration might be an acceptable alternative to inhalation and dermal routes of administration

Specific aim 2: evaluate prechronic toxicity by appropriate route(s) of exposure

- These studies will be conducted beginning with *in utero* exposure
- Heart and skeletal muscle are potential target organs and therefore biomarkers appropriate for monitoring muscle damage and cardiac function will be included
- If ADME studies indicate that absorption through the skin is significant then dermal administration will be used
- If ADME studies indicate that dermal absorption is minimal, then the *in utero* exposure studies will be conducted by oral administration and a separate prechronic dermal study will be conducted since skin is indicated as a potential target organ based on the 2-year studies of o-anisidine and 2-methoxy-5-nitroaniline
- Studies of reproductive toxicity will be included as part of the prechronic evaluation

Key issue: DNA reactivity

- 2-methoxy-4-nitroaniline is positive in some bacterial mutagenicity assays but negative in others
- Three structurally related compounds, 2-methoxy-5-nitroaniline, 2,4-diaminoanisole, and o-anisidine are carcinogens
- If 2-methoxy-4-nitroaniline exhibits significant DNA reactivity, it may not be necessary to conduct a 2-year carcinogenicity study

Specific aim 3: examine DNA reactivity

- Examine bacterial mutagencity in a nitoreductase proficient strain of salmonella
- Evaluate DNA reactivity with comet assay
- Look for formation of DNA adducts in target tissue
- The results of these studies will form the basis for predicting the carcinogenic potential of 2-methoxy-4-nitroaniline

Significance and Outcome

- The proposed studies will provide a complete characterization of the toxicity of 2-methoxy-4-nitroaniline, allow a prediction of carcinogenic potential, and provide sufficient data for dose selection for a 2-year carcinogenicity study should one be necessary