Evidence on the Carcinogenicity of C.I. Disperse Yellow 3

January 25, 2013
Meeting of the Carcinogen Identification Committee

Cancer Toxicology and Epidemiology Section
Reproductive and Cancer Hazard Assessment Branch
Identity of C.I. Disperse Yellow 3

Chemical Structure:

CAS-RN: 2832-40-8

- Molecular Formula: $\text{C}_{15}\text{H}_{15}\text{N}_3\text{O}_2$
- Molecular Weight: 269.30
- Chemical Class: monoazo dye
- Chemical Appearance: powder
- Water Solubility: 1.18 mg/L (at 25°C)
C.I. Disperse Yellow 3 Uses

• A textile dye for coloring nylon, polyvinyl chloride and acrylic fibers, wools, furs, cellulose acetate, polystyrene, and other thermoplastics
• Products include clothing, hosiery, and carpeting
• Dyes in ink products, and in pulp and paper manufacture
Occurrence of C.I. Disperse Yellow 3

- Primarily used in dyeing of synthetic textiles such as yarns, fabrics, and carpets.

- Populations potentially exposed:
  - Workers in synthetic textile manufacturing
    - Example: is one of 39 disperse dyes known to cause contact allergic dermatitis in textile workers.
  - General public using synthetic textiles
    - Example: allergic eczema is associated with nylon hosiery containing C.I. Disperse Yellow 3.
Potential Exposures to C.I. Disperse Yellow 3 in Textile Manufacturing

• Dyeing with C.I. Disperse Yellow 3
  – Yarn stage
    • Batch dyeing machines.
  – Fabric/carpet stage
    • Continuous and batch dyeing machines.

• Handling of dyed yarns, fabrics, and carpets.
  – Exposure to CI DY3 (dermal and respiratory) may be more likely in handling than in dyeing.
Carcinogenicity Studies in Humans

• There are no epidemiology studies of humans with documented exposure to C.I. Disperse Yellow 3.
• There are four epidemiology studies of textile workers with potential exposure.
  – All four studies were of bladder cancer only.
  – All four were case-control design.
  – Three were conducted in Spain, one in New Zealand.
  – All four used interviewer-administered questionnaires to collect exposure data.
  – All four used standard occupation/industry coding. One (Serra et al. 2008) additionally used detailed questions about the textile manufacturing workplace.
**Epidemiology study: Gonzales et al., 1988**

- Case-control study in Spain.
  - Incident cases from **one hospital**.
  - Deceased cases from a **local death registry**.


- 107 hospital and deceased controls

- “Textile dyeing or printing” OR=4.41, 95% CI= 1.15-16.84, based on 8 exposed cases and 3 exposed controls.

- C.I. Disperse Yellow 3 was among 72 dyes mentioned in article.

- Limitation: most subjects deceased (75%) by time of interview, requiring proxy interview (e.g. with spouse).
Epidemiology study: Gonzales et al., 1989

• Case-control study in Spain
  – Incident cases from 12 hospitals in four geographic regions.

• 497 bladder cancer cases (438 male & 59 female) occurred 1985 -1986.

• Two control groups
  – Hospital
  – General population.

• “Textile dyers” OR=1.29, 95% CI= 0.5-3.1, based on 11 exposed cases and 17 exposed controls.

• C.I. Disperse Yellow 3 was not mentioned in the article.
Epidemiology study: Dryson et al., 2008

- Case-control study in New Zealand
  - Cases from nationwide cancer registry.
- 471 controls from general population.
- “Textile products machine operators - textile bleaching, dyeing, and cleaning” OR=0.81, 95% CI=0.19-3.54, based on three exposed cases and 10 exposed controls).
- C.I. Disperse Yellow 3 was not mentioned in the article.
Epidemiology study: Serra et al., 2008

- Case-control design at 18 hospitals in Spain.
- 1,221 controls from the same hospitals.
- Interviewer-administered questionnaires with module designed specifically for textile industry.
- “Winding, warping, and sizing” with “synthetic” materials (OR=15.39, 95% CI=1.89-125.29, based on 11 exposed cases and 1 exposed control).
- “Synthetic” materials 10+ years (OR=2.62, 95% CI 1.14-6.01, based on 21 exposed cases and 9 exposed controls)
- C.I. Disperse Yellow 3 was not mentioned in the article.
Two Carcinogenicity Studies in Rats

- National Toxicology Program (NTP), 1982
- F344 rats (males, females)
  - 50 animals/sex/dose
  - 0, 5,000 or 10,000 ppm in feed for 103 weeks and terminated by 104 weeks.
- Liver and stomach tumors were observed in males
# Feed Studies in Male F344 Rats

<table>
<thead>
<tr>
<th>Organ</th>
<th>Tumor</th>
<th>Dose group (ppm)</th>
<th>Trend test p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>5000</td>
</tr>
<tr>
<td>Liver</td>
<td>Hepatocellular adenoma</td>
<td>1/31</td>
<td>15/45**</td>
</tr>
<tr>
<td></td>
<td>Combined hepatocellular adenoma and carcinoma</td>
<td>2/31</td>
<td>15/45**</td>
</tr>
<tr>
<td>Stomach</td>
<td>Glandular portion:</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Combined adenoma, mucinous adenocarcinoma,</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>and sarcoma</td>
<td>0/30</td>
<td>2/45</td>
</tr>
<tr>
<td></td>
<td>Non-glandular portion:</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Combined squamous cell papilloma and fibrosarcoma</td>
<td>0/30</td>
<td>2/45</td>
</tr>
</tbody>
</table>

Pairwise comparison with controls; * p<0.05, ** p<0.01
Feed Studies in Female F344 Rats

• No treatment-related tumors were observed
Two Carcinogenicity Studies in Mice

- National Toxicology Program (NTP), 1982
- B6C3F₁ mice (males, females)
  - 50 animals/sex/dose
  - 0, 2,500 or 5,000 ppm in feed for 103 weeks and terminated by 104 weeks.
- Lung tumors in males.
- Hematopoietic system and liver tumors in females.
# Feed Studies in Male B6C3F₁ Mice

<table>
<thead>
<tr>
<th>Organ</th>
<th>Tumor</th>
<th>Dose group (ppm)</th>
<th>Trend test p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>2500</td>
</tr>
<tr>
<td>Lung</td>
<td>Alveolar/bronchiolar adenoma</td>
<td>2/47</td>
<td>6/42</td>
</tr>
<tr>
<td></td>
<td>Combined alveolar/bronchiolar adenoma and carcinoma</td>
<td>3/47</td>
<td>7/42</td>
</tr>
</tbody>
</table>

Pairwise comparison with controls; * p<0.05, # p=0.055
# Feed Studies in Female B6C3F₁ Mice

<table>
<thead>
<tr>
<th>Organ</th>
<th>Tumor</th>
<th>Dose group (ppm)</th>
<th>Trend test p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>2500</td>
</tr>
<tr>
<td>Hematopoietic System</td>
<td>Malignant lymphoma</td>
<td>10/50</td>
<td>16/50</td>
</tr>
<tr>
<td></td>
<td>Combined malignant lymphoma and leukemia</td>
<td>10/50</td>
<td>17/50</td>
</tr>
<tr>
<td>Liver</td>
<td>Hepatocellular adenoma</td>
<td>0/50</td>
<td>6/47*</td>
</tr>
<tr>
<td></td>
<td>Hepatocellular carcinoma</td>
<td>2/50</td>
<td>4/47</td>
</tr>
<tr>
<td></td>
<td>Combined hepatocellular adenoma and carcinoma</td>
<td>2/50</td>
<td>10/47*</td>
</tr>
</tbody>
</table>

Pairwise comparison with controls; * p<0.05, ** p<0.01
Genotoxicity in Non-mammalian Species

• *Salmonella* reverse mutations
  - Positive: TA 97, 98, 1537, 1538 strains (+/-S9), and 100 (+S9) strains
  - Negative: TA 100 (-S9) and 1535 (+/-S9) strains

• Chromosomal aberrations in Frog larvae
  - Positive

• Sex-linked recessive lethal mutations in *Drosophila*
  - Negative
**In Vitro Genotoxicity in Mammalian Species**

- **Mouse lymphoma forward mutations**
  - Positive: 2 tests (+S9)
  - Negative: 3 tests (-S9)

- **Sister chromatid exchange in CHO cells**
  - Positive: 1 test (+S9), 1 test (-S9)
  - Negative: 1 test (-S9)

- **Chromosomal Aberrations in CHO cells**
  - Negative (+/-S9)

- **Unscheduled DNA synthesis in rat hepatocytes**
  - Positive (-S9)
In Vivo Genotoxicity in Mammalian Species

Negative
• Micronucleus induction in mouse bone marrow
• DNA damage in rat liver

In Vitro Cell Transformation

Negative
• BALB/c 3T3 mouse cells (-S9)
Pharmacokinetics and Metabolism

• Absorption
  – Dermal absorption is expected
  – Oral absorption is inferred
  – Inhalation unknown

• Azoreduction
  – Azo dyes undergo reductive cleavage of the azo bond and form aromatic amine metabolites
  – Metabolites: 4-Aminoacetanilide and 2-Amino-p-cresol
Proposed Mechanism Of Azo Reduction

Adapted from Levine (1991)
C.I. Disperse Yellow 3 and its Expected Metabolites

C.I. Disperse Yellow 3

4-Aminoacetanilide + 2-Amino-p-cresol
# Genotoxicity of Metabolites

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Genotoxicity</th>
<th>In vitro</th>
<th>In vivo</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-Aminoacetanilide</td>
<td><em>Salmonella</em> reverse</td>
<td>Mouse bone marrow; chromosomal</td>
<td></td>
</tr>
<tr>
<td></td>
<td>mutation</td>
<td>aberrations</td>
<td></td>
</tr>
<tr>
<td>2-Amino-(p)-cresol</td>
<td><em>Salmonella</em> reverse</td>
<td></td>
<td>Not tested</td>
</tr>
<tr>
<td></td>
<td>mutation; mouse</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>lymphoma forward</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>mutation</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Structurally Related Chemicals

C.I. Disperse Yellow 3

Azobenzene

\[
\begin{align*}
\text{Prop 65 listed} \\
\text{IARC 3}
\end{align*}
\]

\[
\begin{align*}
\text{Prop 65 listed} \\
\text{IARC 2B}
\end{align*}
\]

\[
\begin{align*}
\text{Prop 65 listed} \\
\text{IARC 2B}
\end{align*}
\]

\[
\begin{align*}
\text{Prop 65 listed} \\
\text{IARC 2B}
\end{align*}
\]

Oil Orange SS

\[
\begin{align*}
\text{Prop 65 listed} \\
\text{IARC 2B}
\end{align*}
\]
Structurally Related Chemicals

C.I. Disperse Yellow 3

4-Aminoacetanilide

2-Amino-p-cresol

2,4-Diaminotoluene

2-Aminotoluene

Phenacetin

Prop 65 listed
IARC 2B

Prop 65 listed
IARC 1

Prop 65 listed
IARC 2A
## Carcinogenicity of Structurally Related Chemicals

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Liver</th>
<th>Other Sites</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mice</td>
<td>Rats</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C.I. Disperse Yellow 3</td>
<td>F</td>
<td>M</td>
</tr>
<tr>
<td></td>
<td>M (hematopoietic system)</td>
<td>M (Stomach)</td>
</tr>
<tr>
<td></td>
<td>M (lung)</td>
<td></td>
</tr>
<tr>
<td>Azobenzene</td>
<td>M</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M (spleen)</td>
<td></td>
</tr>
<tr>
<td>p-Aminoazobenzene</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td></td>
<td>M (skin)</td>
<td></td>
</tr>
<tr>
<td>o-Aminoazotoluene</td>
<td>MF</td>
<td>MF</td>
</tr>
<tr>
<td></td>
<td>MF (lung)</td>
<td>MF (lung)</td>
</tr>
<tr>
<td></td>
<td>F (soft tissues)</td>
<td></td>
</tr>
<tr>
<td>Oil Orange SS</td>
<td>MF</td>
<td>MF (bladder)</td>
</tr>
<tr>
<td></td>
<td>M (intestinal)</td>
<td></td>
</tr>
<tr>
<td>2,4-Diaminotoluene</td>
<td>F</td>
<td>MF</td>
</tr>
<tr>
<td></td>
<td>M (kidney, skin)</td>
<td>M (kidney, skin)</td>
</tr>
<tr>
<td></td>
<td>F (mammary, lymphoma)</td>
<td></td>
</tr>
<tr>
<td>2-Aminotoluene</td>
<td>F</td>
<td>MF (blood vessels)</td>
</tr>
<tr>
<td></td>
<td>M (abdominal, scrotum, skin, spleen)</td>
<td>M (abdominal, scrotum, skin, spleen)</td>
</tr>
<tr>
<td></td>
<td>F (mammary, bladder, spleen)</td>
<td></td>
</tr>
<tr>
<td>Phenacetin</td>
<td>M</td>
<td>MF (urinary tract)</td>
</tr>
<tr>
<td></td>
<td>MF (urinary tract, nasal cavity)</td>
<td></td>
</tr>
</tbody>
</table>
Possible Mechanisms of Action

- Genotoxicity
  - Mutagenicity and clastogenicity by the parent compound and metabolites
  - Structural similarity with carcinogenic monoazo compounds and related aromatic amines that are genotoxic
Summary of Human Evidence

• Four case-control studies of bladder cancer risk among textile workers.
• Exposure-related limitations of all four studies:
  – C.I. Disperse Yellow 3 was just one of many disperse dyes used. C.I. DY3 was mentioned in only one study.
  – No exposure measures or cancer risk results for specific dyes.
• Two of the four reported significant associations for jobs with potential exposure to C.I. Disperse Yellow 3.
• The studies are inadequate to assess the relationship between C.I. Disperse Yellow 3 exposure and cancer risk.
### Summary Of Animal Evidence

#### F344 rats

- **Males:**
  - Benign and combined malignant / benign liver tumors
  - Rare stomach tumors

- **Females:**
  - No treatment-related tumors

#### B6C3F1 mice

- **Males:**
  - Benign and combined malignant/benign lung tumors

- **Females:**
  - Hematopoietic system tumors
  - Benign and combined malignant / benign liver tumors
Summary Of Other Relevant Evidence

• *In vitro* genotoxicity in a variety of systems
• Metabolism to genotoxic metabolites
• Structurally similar to other carcinogens