CI Solvent Yellow 14 shows activity in the bone marrow micronucleus assay in both the rat and mouse

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Introduction

CI Solvent Yellow 14 is an azo dye based on aniline and 1-amo-2-hydroxynaphthalene (Figure 1). In an NTP study (NCI, 1982), CI Solvent Yellow 14 was reported to be carcinogenic in the rat, but not the mouse, causing an increase in neoplastic nodules in the liver of the rats at the highest dose of 500 p.p.m. A number of studies have been conducted on CI Solvent Yellow 14 to examine for genotoxicity in vitro, including gene mutation, chromosomal aberration, sister chromatid exchange (SCE) and DNA repair end points. The chemical has been reported to produce positive responses in the Ames test (Cameron et al., 1987; Zeiger et al., 1988), L5178Y mutation assay (Cameron et al., 1987) and Chinese hamster ovary (CHO) SCE assays (Ivett et al., 1989). Negative results were reported for a CHO chromosome aberration assay (Ivett et al., 1989) and a rat hepatocyte DNA repair assay (Kornbrust and Barfnecht, 1985). Positive Ames results were seen in Salmonella strains TA98 and TA1538 in the presence of S9, a profile consistent with aromatic amines.

Studies to examine for the genotoxicity of CI Solvent Yellow 14 in vivo have employed the liver unscheduled DNA synthesis (UDS) and DNA repair assay and the bone marrow micronucleus assay. The liver UDS assay was conducted in the rat and gave a weakly positive result at a dose of 500 mg/kg (Kornbrust and Barfnecht, 1985) or a negative result at doses up to 1000 mg/kg (Mirsalis et al., 1989; Westmoreland and Gatehouse, 1991). The bone marrow micronucleus assay has been conducted in both the rat and mouse (to dose levels of 1000 and 2000 mg/kg respectively) and reported as positive in the rat but negative in the mouse (Westmoreland and Gatehouse, 1991). In view of the importance of the in vivo genotoxicity profile of a chemical when assessing possible hazard to man, and the conflicting literature findings, CI Solvent Yellow 14 has been examined in the rat and mouse bone marrow micronucleus assays and the rat liver UDS assay (at multiple sampling times), extending the dose levels up to limit doses values of 5000 and 2000 mg/kg respectively.

Materials and methods

Animals

Male C57BL/6j, BL10/Alpk mice (aged 6–12 weeks) and male Alpk: AP/SD rats (5–9 weeks old) were used for the micronucleus assay, with male Alpk: AP/SD rats (6–8 weeks old) used for the liver UDS assay. All animals were supplied by RBU, Alderley Park, Cheshire, UK. Animals were given food (PCD or CT1; Special Diets Services, Witham, UK) and water ad libitum.

Chemicals

CI Solvent Yellow 14 was supplied by Zeneca Specialties (Manchester, UK) as a yellow/orange powder with an analysed purity of 96% w/w. The material was stored in the dark at ambient temperature until required and was tested as a suspension in corn oil (prepared by homogenization) with no correction for purity. Cyclophosphamide and dimethylnitosamine (DMN) were from Sigma Chemical Co Ltd (Poole, UK) and were dissolved in physiological saline.

6-p-Dimethylaminoazobenzene (6BT) was synthesized at Central Toxicology Laboratory and was prepared in corn oil with homogenization. Dosing preparations were administrated at a volume of 20 ml/kg bodyweight for the micronucleus assays and 10 ml/kg bodyweight for the UDS assay.

Bone marrow micronucleus test

An initial dose ranging study was conducted to assess the toxicity of CI Solvent Yellow 14. For the main studies, groups of five animals were given a single oral dose of test compound or control substance and killed after 24 or 48 h by exposure to halothane (Fluothane; Zeneca Pharmaceuticals) or a rising concentration of CO2 followed by cervical dislocation. Bone marrow smears were prepared (Sheldon et al., 1987) and stained with polychrome Methylene Blue and eosin (mouse) or haematoxylin and eosin (rat) (Pascoe and Gatehouse, 1986). All slides were coded before scoring. Initially 1000, but eventually up to 6000 polychromatic erythrocytes (PCEs) per animal were examined for micronuclei, and 1000 erythrocytes per animal to determine the percentage of polychromatic erythrocytes. A one-sided Student’s t-test on transformed data (natural logarithmic transformation) was used to compare test and vehicle control groups.

Fig. 1. CI Solvent Yellow 14.
controls were seen at both dose levels at the 24 h sampling time, and at the higher dose level at the 48 h sampling time. As with the rat, comparison of the percentage of PCEs showed no significant differences between the CI Solvent Yellow 14 and vehicle control animals (data not shown).

Liver UDS assay

No signs of toxicity were observed in rats dosed with 2000 mg/kg of CI Solvent Yellow 14 in a dose ranging study, and this dose level was therefore used for the assessment of UDS, along with 1000 mg/kg as a lower dose level. Animals dosed with CI Solvent Yellow 14 had mean net nuclear grain counts (nuclear grain count – cytoplasmic grain count) of <0, and so showed a clear negative response in this assay (Table III). There was also no increase in the mean number of cells in repair (cells with a net nuclear grain count of >5).

Discussion

CI Solvent Yellow 14 was reported as carcinogenic in an NTP bioassay in the rat, but non-carcinogenic in the mouse. The lesion in the rat was an increase in 'neoplastic nodules' at the highest dose of 500 p.p.m. This term was originally adopted by pathologists to cover a range of focal lesions from hyperplasia to benign neoplasia, but is now no longer in general use. Evaluation of such lesions by current criteria would most likely lead to classification as benign liver tumours, i.e. hepatocellular adenomas. In the NTP study there was no increase in malignant liver tumours, and the evidence for carcinogenicity therefore rests with an increase in benign tumours only. In such cases, the profile of genotoxicity for the chemical, and in particular the in vivo genotoxicity, can contribute significantly to the overall assessment of carcinogenic hazard. Previous reports in the literature were conflicting in indicating that CI Solvent Yellow 14 was positive in the bone marrow micronucleus assay in the rat but negative in the mouse (Westmoreland and Gatehouse, 1991), and with results showing both a weak positive (Kornbrust and Barfnecht, 1985) and a negative (Mirsalis et al., 1989; Westmoreland and Gatehouse, 1991) result in the rat liver UDS assay. The present study has extended the dose levels tested in these assays, up to limit doses of 5000 mg/kg for the micronucleus assay, and 2000 mg/kg for the liver UDS assay.
CI solvent yellow 14 in rat and mouse micronucleus assays

Table II. CI Solvent Yellow 14: evaluation in the mouse bone marrow micronucleus assay

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (mg/kg)</th>
<th>Mean incidence of MPE/1000 PCE ± SD</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>24 h</td>
</tr>
<tr>
<td>Experiment 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn Oil</td>
<td>20 ml/kg</td>
<td>1.8 ± 1.9 (2.4 ± 0.4)</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>65</td>
<td>17.0 ± 6.3 (14.9 ± 3.1)</td>
</tr>
<tr>
<td>CI Solvent Yellow 14</td>
<td>5000</td>
<td>2.8 ± 2.3 (2.9 ± 1.0)</td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn Oil</td>
<td>20 ml/kg</td>
<td>3.8 ± 1.6 (2.3 ± 0.4)</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>65</td>
<td>27.2 ± 5.4 (21.7 ± 3.1)</td>
</tr>
<tr>
<td>CI Solvent Yellow 14</td>
<td>2000</td>
<td>5.4 ± 2.7 (3.8 ± 1.0)</td>
</tr>
<tr>
<td>CI Solvent Yellow 14</td>
<td>5000</td>
<td>7.0 ± 3.1 (4.4 ± 1.4)</td>
</tr>
</tbody>
</table>

MPE, micronucleated polychromatic erythrocyte; PCE, polychromatic erythrocyte. MPE values are for 1000 PCEs per animal except for values in parentheses which are for 6000 PCEs per animal. All means based on five animals. *P < 0.01; bP < 0.05.

In both the mouse and the rat there were no signs of overt toxicity, but there was evidence of absorption of CI Solvent Yellow 14 in the form of orange-coloured urine. This is in contrast to the study of Westmoreland and Gatehouse (1991) who found no evidence for absorption of CI Solvent Yellow 14 in mice. The results from the present study have confirmed that CI Solvent Yellow 14 is positive in the bone marrow micronucleus assay, with increases in MPE values in both the rat and the mouse. The increases seen following an initial assessment of 1000 PCEs were not large however, and the number of PCEs scored was therefore increased to 6000 per animal in order to be confident of the data representing an accurate sampling and assessment of the bone marrow smears. In addition, two independent studies were conducted in both
the rat and the mouse to examine the reproducibility of the effect. As expected, increasing the number of PCEs to this extent reduced the variability observed between experiments. The increases in MPE seen in the rat, were indeed reproducible, were clearly above the vehicle control values, and were consistent with previous literature data. The increases in MPE over the control values seen in the mouse were less than the rat, but were nevertheless reproducible in the two independent experiments and indicated that CI Solvent Yellow 14 is not uniquely active in the rat in the micronucleus assay, as initially proposed by Westmoreland and Gatehouse (1991). It must be noted that the strain of mouse used in this study is not the same as the CRH strain used by Westmoreland and Gatehouse (1991), and the control frequency of micronuclei is different between the two strains. Such strain differences may be important in the detection of weak effects in treated animals. The increases in micronuclei observed in the mouse at the 2000 mg/kg dose level (the limit dose to be recommended in the forthcoming revised OECD guideline for the micronucleus assay) were small, but were retained and achieved statistical significance after scoring an increased number of PCEs. Such an increase alone would be taken as indicating a possible effect of the compound, but would clearly indicate only weak activity.

The negative response in the rat liver UDS assay at doses up to a limit of 2000 mg/kg confirms the previous observation made at lower dose levels by Westmoreland and Gatehouse (1991) and does not support the weak positive result of Kornbrust and Barfnecht (1985). However, the rat liver UDS assay has shown to be sensitive to a number of genotoxic aromatic amines or azo hepatocarcinogens and was therefore considered appropriate for such an evaluation for CI Solvent Yellow 14, as opposed to other assays (e.g. rat liver micronucleus assay). In view of the negative response observed, the contribution, if any, of the genotoxicity expressed by the bone marrow micronucleus assay to the formation of liver nodules on chronic administration of the compound, is unclear.

The purity of the sample of CI Solvent Yellow 14 used in this study was 96%, with hplc analysis indicating a number of impurities present at a low level. These impurities have not been characterized. The sample purity was, however, similar to that reported for the NTP bioassay sample (94.1%), and the use of the bioassay sample, together with an independent sample from Sigma by Westmoreland and Gatehouse (1991), with similar results in the rat to those from the present study, suggests that the effects seen are likely to be caused by the parent material.

The genotoxicity profile of CI Solvent Yellow 14 is one of genotoxic activity in vivo, and with a profile in the Ames test consistent with an aromatic amine. In vitro, there have been some conflicting reports of activity, however the data from the present studies (considering the rat and mouse together) confirm genotoxic activity in the bone marrow micronucleus assay. The findings from these genotoxicity assays, together with the observation of an increase in neoplastic nodules in the NTP rat bioassay, has led members of the Ecological and Toxicological Association of Dyes and Organic Pigments Manufacturers (ETAD) to include statements on these effects in their Safety Data Sheets and to prepare a proposal for the labelling of CI Solvent Yellow 14 for the consideration of the European Union.

References
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