Nitrosatable amines and amine derivatives have an important role in many industrial processes. Handling, production, and use of these chemicals may result in exposure to the corresponding N-nitroso products, which could have mutagenic or carcinogenic activity (1). Cutting fluids are widely used to reduce the temperature of the metal–tool interface during metal cutting or grinding and have been found to contain some N-nitrosamines. N-nitosodiethanolamine (NDELA) is the most common N-nitrosoamine in cutting fluids, which are formulated with ethanolamine and nitrite, the precursors of NDELA (2). NDELA is a strong animal carcinogen (3–6), a mutagen in the Ames test after activation with alcohol dehydrogenase (7,8), and a potent inducer of DNA damage in primary hepatocytes in vitro (7,9). For these reasons NDELA could represent a health risk for workers in the metal industry, who are exposed by direct skin contact or by inhalation of the oil mist.

Following an integrated environmental/biological monitoring approach previously used by our research group in different working environments (10) and drawing from published studies of NDELA monitoring in metal industries (11–13), we evaluated exposure of metal workers to NDELA and other potentially genotoxic compounds found in cutting fluids. We analyzed cutting fluids sampled in different metal factories in central Italy for nitrite and NDELA content and mutagenic activity. Biological monitoring was carried out on workers who used cutting fluids that were positive for NDELA by analyzing NDELA content of urine, assessing mutagenicity, determining thioether and D-glucaric levels, and determining sister chromatid exchanges (SCE) in peripheral blood lymphocytes. This paper deals only with the environmental monitoring phase. Biological monitoring data are still being gathered.

We collected new and used cutting fluid samples (N = 63) during working hours in several metal factories, took them to the laboratory, and tested them for nitrite content by a rapid and semiquantitative method. The samples that were positive for nitrite, a NDELA precursor, were analyzed for nitrite content by a quantitative method, NDELA content, and mutagenic activity. Nitrite-negative samples were analyzed only for mutagenicity to detect mutagens other than NDELA. The scheme of the environmental monitoring approach followed is given in Figure 1.

Nitrite detection was first performed using a rapid and semiquantitative screening method (Merrickquant, nitrite test, Merck, Germany), where test strips were immersed in the emulsions. We performed the quantitative nitrite determination only on nitrite-positive samples, using an analytical procedure that is an adaptation of standard nitrite/nitrate determination to a continuous flow analyzer. A 1-ml aliquot was used for the determination of nitrite using a dual-channel, continuous-flow nitrite/nitrate analyzer (14). Samples positive for nitrite were combined with 1–2 g/100 ml of NaOH and analyzed for NDELA determination. We treated 0.5 g of cutting fluids with about 2 g sulfamic acid to destroy the nitrite and added distilled water to yield 15 ml. Extraction was done using a silica column containing a layer of ascorbic acid (to trap ethanolamines) and a layer of potassium carbonate (to trap ascorbic acid), eluted with 50 ml ethyl formate containing 2% methanol. We evaporated extracts to dryness under a stream of nitrogen and reacted the residue with 0.3 ml of a silylating agent (N-methyl-N-trimethylsilylheptafluorobutyramide) at 80°C for 2 hr. NDELA quantification was carried out by gas chromatography/chemiluminescence detection (TEA 502) (12,13). The gas chromatograph conditions were: injector, 200°C; on-column injection; column, 0.635 cm o.d., 0.2 cm i.d. X 140 cm silanized boro-silica glass filled with 6% OV275 on Volashop A2 (Merk, Germany); oven, initial temperature 110°C, 5 min, temperature program 10°C/min, final temperature 220°C, 5 min. We assessed the mutagenic activity of the samples directly, as in toto samples, and as basic fractions, presumably containing nitrosamines. The in toto samples were filtered sterilized, added with Tween 80 and tested at increasing doses (up to 150 µl/plate), with the plate-test version of the Ames test (15), according to the Hermann.

**Figure 1.** Scheme of the analysis of cutting fluids (N = 63) sampled in Italian metal factories.
Table 1. Concentrations of nitrite, N-nitosodiethanolamine (NDELA), and mutagenicity in new or used cutting fluids found to contain nitrite by means of a rapid and semiquantitative method

| Factory | Sample | Used/ New | Nitrite (mg/kg) | NDELA (mg/kg) | Mutagenicity, TA100 in toto | Mutagenicity, TA100 Basic fractions |
|---------|--------|-----------|----------------|---------------|----------------------------|----------------------------------|
|         |        |           |                |               | -S9 | +S9 | +S9 | +ADH |
| A       | A1     | New       | 13             | 5.0           | -   | -   | +   | -    |
|         | A2     | Used      | 55             | 24.0          | -   | -   | +   | -    |
|         | A3     | Used      | 2540           | 1900          | -   | +   | +   | -    |
| B       | B1     | New       | 2600           | 17.9          | -   | -   | +   | -    |
|         | B2     | Used      | 1800           | 9.1           | -   | -   | +   | -    |
| C       | C1     | New       | 6500           | 5.3           | -   | -   | +   | -    |
|         | C2     | Used      | 5700           | 6.5           | -   | -   | +   | -    |
| D       | D1     | New       | 2              | 0.4           | -   | -   | +   | -    |
|         | D2     | Used      | 4              | 0.3           | -   | -   | +   | -    |
| E       | E1     | New       | 70             | 2.4           | -   | -   | +   | -    |
|         | E2     | Used      | 10             | 0.3           | -   | -   | +   | -    |
| F       | F1     | Used      | 60             | 1.0           | -   | -   | +   | -    |
| G       | G1     | New       | 19,600         | 30.7          | -   | -   | +   | -    |

See text for details on methods. Mutagenicity results were obtained with the Ames plate test (19) for the in toto samples and with the preincubation modification of the Ames test (17) for the basic fractions, using TA100 strain with either a post-mitochondrial liver fraction obtained from Aroclor-induced rats (+S9 mix), or alcohol dehydrogenase (ADH) activation. These data are expressed only qualitatively (see Table 2 for dose-response results; the samples showing dose-response results and a mutagenicity ratio > 2 were considered mutagenic). All the nitrite-negative samples were nonmutagenic.

Table 2. Mutagenicity of basic extracts of some nitrite-positive cutting fluids

| Samples | Dose (ml/plate) | Revertants/plate | Mutagenicity ratio | Net revertants/ml |
|---------|----------------|------------------|--------------------|-------------------|
| A1      | 6.25           | 134              | 1.2                | 6                 |
|         | 12.50          | 213              | 1.9                | 12                |
|         | 25.00          | 246              | 2.2                | 2.9               |
|         | 37.50          | 287              | 3.4                | 3.7               |
| A2      | 6.25           | 143              | 1.4                | 4                 |
|         | 12.50          | 227              | 2.3                | 8                 |
|         | 25.00          | 287              | 2.9                | 2.9               |
|         | 37.50          | 392              | 3.4                | 4.4               |
| A3      | 6.25           | 119              | 1.2                | 2                 |
|         | 12.50          | 218              | 2.2                | 8                 |
|         | 25.00          | 280              | 2.8                | 3                 |
|         | 37.50          | 127              | 3                 | 3                 |
| B1      | 6.25           | 112              | 1.0                | 2                 |
|         | 12.50          | 214              | 1.8                | 12                |
|         | 25.00          | 392              | 3.4                | 3.4               |
|         | 37.50          | 119              | 1.2                | 1                 |
| B2      | 6.25           | 296              | 2.5                | 5                 |
|         | 12.50          | 514              | 4.4                | 20                |
|         | 25.00          | 608              | 5.2                | 5.2               |
|         | 37.50          | 127              | 3                 | 3                 |
| C1      | 6.25           | 181              | 2.2                | 3                 |
|         | 12.50          | 238              | 3.6                | 11                |
|         | 25.00          | 364              | 4.5                | 4.5               |
|         | 37.50          | 280              | 3                 | 3                 |
| C2      | 6.25           | 1184             | 14.4               | 176               |
|         | 12.50          | 2280             | 27.8               | 27.8              |
|         | 25.00          | 420              | 33                 | 33                |
|         | 37.50          | 600              | 3                  | 3                 |
| G1      | 1.25           | 102              | 1.1                | 3                 |
|         | 2.50           | 108              | 1.2                | 3                |
|         | 6.25           | 296              | 3.0                | 3                 |
|         | 12.50          | 760              | 5                 | 5                 |

The results were obtained from preincubation assay with S. typhimurium TA98 and TA100 strains, ± S9 or as basic fractions (TA100 + S9 or + alcohol dehydrogenase). In contrast, 61% of the nitrite- and NDELA-positive samples (8 samples, 12.7% of the total samples) were mutagenic to TA100 + S9 in the basic extracts (Table 2). When tested in toto, only one sample was mutagenic to TA100 + S9. It must be pointed out that even though nitrates are mutagenic to TA100 + S9, all the samples were negative when tested using TA100 + S9 or TA98 + S9.

Activation by alcohol dehydrogenase gave positive results only in the A3 sample, which had the highest concentration of NDELA (1900 mg/kg; Fig. 2). The negative results for the other samples could be due to the lower NDELA concentrations. Sample A3 was also the only positive sample when tested in toto with TA100 + S9.

The mutagenicity ratio and specific mutagenicity data revealed extremely high activity for sample C2 and high activity for samples B2 and G1. The highest doses tested (37.5 ml/plate) of all the samples were toxic to bacteria.
It should be pointed out that mutagenicity was found among the samples with the highest NDELA content (25 mg/kg). However, because tests with the alcohol dehydrogenase activating system were positive for only one sample, mutagenicity is probably due to other basic compounds different from NDELA. These unknown mutagens were not found in the nitrite-negative samples. Thus, unknown mutagenic substances are present in the basic extracts of these products. Identification of these compounds could lead to better estimation of the health hazards associated with exposure to cutting fluids.

In conclusion, preventive programs based on nitrite screening in the field, together with mutagenicity testing of cutting fluids, are important for identifying samples that could potentially contain NDELA and other unknown mutagens. All cutting fluids should be monitored for nitrite content before use in factories by using rapid nitrite screening tests, and the positive samples should be rejected or, as mandated in some countries, the nitrite in cutting fluids should be banned or restricted to those products that do not contain amine precursors. However, the presence of unknown potential mutagens in these fluids should be monitored using short-term mutagenicity tests both in in toto samples and in the basic extracts. All these measures should be carried out along with more general preventive measures in the workplace to minimize the health hazards associated with occupational exposure to cutting fluids. These results will be completed with further data, obtained from the biological monitoring program carried out among the workers of the metal factories where NDELA-positive cutting fluids are used.

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