STUDIES OF ATRAZINE EFFECTS ON DNA IN MICE
BY CHROMOSOMAL ABERRATION TEST AND
AGAROSE GEL ELECTROPHORESIS ASSAY

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ABSTRACT

The herbicide atrazine was tested for its ability to induce chromosomal aberrations in mouse bone marrow. Also, the alkaline single cell gel electrophoresis was used to measure DNA strand breaks in both kidney and liver tissues. Mice were given orally a single dose of 28.125, 56.25, 112.5 and 225 mg/kg body weight (1/64, 1/32, 1/16 and 1/8 of LD₅₀) and repeated daily doses of atrazine. Mice were sacrificed 24 hrs after the last dose. A slight increase in chromosome aberration was observed for the vehicle group (corn oil) compared with the negative control (untreated mice). A significant increase in the percentage of chromosome aberrations in bone marrow was observed after oral administration of atrazine at 56.25, 112.5, and 225 mg/kg body weight for 24 hours. The mean of mitotic index induced by this herbicide did not differ significantly from that observed in the negative control. Atrazine-induced numerical chromosomal aberrations in 24h treatment with a dose-response relationship. Moreover, the present data clearly indicate that atrazine possesses the potential, at least to a limited extent to cause alterations in the cellular DNA in both kidney and liver tissues. In conclusion, the induction of chromosome aberrations and DNA damage following exposure to atrazine indicates a potential for clastogenicity.

INTRODUCTION

Atrazine (2-chloro 4 (ethylamino) 6-(isopropylamino) 1,3,5 triazine) is one of the most commonly used herbicides in North America is frequently detected in ground and surface water (Dooley et al., 2006). Atrazine is the most heavily applied agricultural pesticide for crop production in the United States. Both animal and human studies have suggested that atrazine is possibly carcinogenic, but results have been inconclusive (Rusiecki et al., 2004).

The three herbicides atrazine, cyanazine or simazine significantly increased the rate of apparent dominant lethals, x-linked recessive lethals and x or y loss after treatment by larval feeding in Drosophila melanogaster (Murnik and Nash, 1977). Nishi et al. (1979) suggested that maleic hydrazide and its salts had weak inducibility of cytotoxicity and positive cytogenetic effects on Chinese hamster V79 cells in vitro. However, trifluralin and simazine, but not atrazine, significantly increased sister chromatid exchanges per cell with the highest concentrations in human lymphocyte (Ghiazza et al., 1984). Meschini et al. (1988) showed that maleic hydrazide was genotoxic in mammalian cells in vitro but apparently not in vivo. In 1992, Meisner et al. demonstrated dose-related cytogenetic damage not associated with mitotic inhibition or cell death due to the alachlor-atrazine combination suggesting an additive model in vitro.

Roloff et al. (1992) showed little chromosome damage in human lymphocytes exposed in vitro to either linuron or atrazine, whereas significant chromosome damage was observed in lymphocytes simultaneously exposed to linuron and atrazine. In 1993, Kligerman et al. found a concentration related increase in sister-chromatid exchange in splenocytes of rat and mouse exposed through their drinking water to a mixture of pesticides (aldicarb, atrazine, dibromochloropropane, 1,2-dichloropropane, ethylene dibromide and simazine) that simulated contaminated groundwater in California. Exposing human lymphocyte cultures to concentrations of N-nitrosoatrazine (NNAT) as low as 0.0001 microgram/ml results in significant elevations in chromosome breakage as well as an increased mitotic index (Meisner et al., 1993). Moreover, Lioi et al. (1998) observed a dose-related increase in the percent of aberrant cells, an increase of sister chromatid exchanges/cells, a significant reduction of the mitotic index, and increase of glucose-6-
phosphate dehydrogenase (G6PD) enzyme activity in human peripheral lymphocytes exposed in vitro to glyphosate, vinclozolin, atrazine and DPX-E9636. In contrast, Ribas et al. (1998) concluded that atrazine was able to exert a weak cytotoxic effect using sister-chromatid exchanges, chromosome aberrations and micronuclei in cultured human peripheral blood lymphocytes.

Taets et al. (1998) observed karyotype damage in Chinese hamster ovary (CHO) cells with a combination of atrazine and simazine or atrazine and cyanazine as measured by flow cytometry. Kligerman et al.(2000) showed that atrazine, simazine, and cyanazine were found to produce any significant increases in sister chromatid exchanges or chromosomal aberrations in human lymphocyte cultures at the limits of solubility and toxicity. Garaj-Vrhovac and Zeljezic (2001) showed an increased number of chromosomal aberrations, sister chromatid exchange (SCE) frequency, micronucleus (MN) frequency and values of Comet assay parameters in Croatian workers occupationally exposed to a complex mixture of pesticides containing atrazine, alachlor, cyanazine, 2,4- dichlorophenoxyacetic acid and malathion. In addition, at extremely low levels, atrazine was found to damage animals chromosomes in cell culture as mentioned by Rayburn et al. (2001).

The mean value of sister chromatid exchange, chromatid and chromosome breaks, micronuclei and DNA migration in workers occupationally exposed to a mixture of pesticides (atrazine, alachlor, cyanazine, 2,4- dichlorophenoxyacetic acid and malathion) were significantly higher in the comparison with the control group (Garaj-Vrhovac and Zeljezic, 2002 and Zeljezic and Garaj-Vrhovac, 2002). Moreover, Malik et al. (2004) demonstrated a dose dependent increase of SCE in cultured lymphocytes after exposure to atrazine. In 2006, Bouilly et al. showed that the chromosome pairs (1,5,9 and 10) were affected by the loss of 1 chromosome (61%, 15%, 42% and 42% respectively) in the progeny of oysters contaminated by atrazine using the restriction enzyme (HaeIII) digestion chromosome banding.

On the other hand, atrazine affected the circadian rhythms of RNA; DNA and total protein differently, in relation to the specific endocrine tissue of male rats (Nicolau and Socoliuc, 1980). Pino et al. (1988) detected DNA breaks in cell suspensions obtained from stomach, kidney and liver, but not in those from lung by the DNA alkaline elution technique in rats given orally a single high dose or repeated daily doses of atrazine. In 1997, Clements et al. indicated that the herbicide atrazine currently used in Southern Ontario (Canada) is capable of inducing DNA damage in tadpoles using the alkaline single cell gel DNA electrophoresis (SCGE) or Comet assay. In contrast, no significant increase in maleic hydrazide- induced DNA damage, as measured by the Comet assay, could be demonstrated in either leaves of tobacco (Nicotiana tabacum var.xanthi) or roots of field beans, Vicia faba (Gichner et al., 2000).

Moreover, the Comet assay on nuclei from Tradescantia staminal hairs is a useful tool to monitor genotoxic agents such as maleic hydrazide as concluded by Alvarez-Moya et al. (2001). Tennant et al. (2001) indicated that atrazine was induced a small dose-related increase in DNA damage in mice using the alkaline single cell gel electrophoreses (SCGE) assay. Zeljezic and Garaj-Vrhovac (2001) found statistically increased levels of DNA damage in the Comet assay in terms of tail length and tail moment after the period of high exposure to a mixture of pesticides. Undeger and Basaran (2002) observed the DNA damage in lymphocytes of pesticide- exposed workers was significantly higher than that in the controls (P< 0.001).

Zeljezic and Garaj-Vrhovac (2004) found statistically significant increase of tail length for the blood samples, kidney, liver, bone marrow and spleen examined in mice treated with two pesticide formulations (Bravo and Gesaprim) containing alachlor and atrazine as active ingredients compared to the control. For both pesticides DNA of kidney and liver showed largest increase in migration. Chang et al. (2005) stated that both alachlor and atrazine were showed dose related increases in DNA damage in blood cells of common carp (Cyprinus carpio) using the single – cell gel electrophoresis method at environmentally relevant concentrations, (<100ppb). In addition, Monroy et al. (2005) showed DNA damage in normal human cells (GM38) and human fibrosarcoma (HT1080) cells at glyphosate concentrations of 4.0-6.5 and 4.75-5.75, mM respectively using the Comet assay. Recently, Bhalli et al. (2006) indicated that occupational exposure to pesticides causes DNA damage with the single cell gel electrophoresis (SCGE) assay or Comet assay.

In the present study, the genotoxic potential of atrazine, as revealed by bone marrow chromosome aberration and DNA amount using the agarose gel electrophoresis assay in both liver and kidney tissues of mice is reported.

**MATERIALS AND METHODS**

**Animals:**

Male mice, aged 10-12 weeks and weighing 25-30g were used. All animals were kept under the same laboratory conditions of temperature (20±2°C) under 12 hrs light/dark cycle and 50% humidity. The mice were received standard laboratory chow and water ad libitum. Six animals were used for each treatment and control group.

**Test agent:**

Atrazine (CAS No 1912-24-9, purity 97.7%) with the empirical formula C₈ H₁₄ CIN₅) was obtained from Sigma Chemical Company.
Doses:

The herbicide was tested in male mice in four doses approximately 1/64, 1/32, 1/16, and 1/8 of published LD_{50} values (Gebel et al., 1997). Atrazine was dissolved in corn oil and administered orally into mice at dose levels of 28.125, 56.25, 112.5 and 225 mg/kg body weight. Gavage volumes were 0.1 ml per 10 g body weight. For negative control, corn oil only was used.

Chromosomal aberrations (CAs) assay:

Mice were administered with a single dose of 28.125, 56.25, 112.5 and 225 mg/kg body weight via the oral gavage route for 24 hours and chromosome preparations were made as described earlier (Agarwal et al., 1994). Mice were injected (i.p.) with colchicine (4 mg/kg) 2 hrs prior to sacrifice. The bone marrow cells were aspirated in buffer solution and centrifuged at 1000 rpm for 10 minutes. The pellets obtained were mixed in aqueous solution of KCl and left for 20 min at 37°C. Cells were refluxed, fixed in cold Carnoy's fluid (methanol – glacial acetic acid, 3:1) and dropped on clean chilled slides. Finally, slides were air dried and stained with 4% Giemsa.

Mitotic index (M.I.) was calculated from 1000 cells/animal and expressed in percentage (Rios et al., 1995).

\[ \text{Mitotic index (M.I.)} = \frac{\text{number of metaphases}}{1000 \text{ cells}} \times 100 \]

Statistical analysis:

Data from control and treated animals for mitotic preparation were evaluated with Student's t-test at p<0.05 (Snedecor and Cochran, 1980).

Preparation of genomic DNA from mouse tissue:

Mice injected with atrazine were sacrificed 24 hrs after the last injection and both liver and kidney tissues were removed for the determination of DNA content.

Prepare the tissues:

1- Excision and immediately mince of the tissue and freeze in liquid nitrogen.

2- Grind 250 mg tissue with hemogenizer. Suspended in 2.5ml digestion buffer (1MTris-HCl, 1mM EDTA, 1% sodium dodecyl sulphate (SDS), 10 mg/ml pancreatic RNase or nucleic acid purification grade lysis).

DNA extract:

1- Samples were incubated, shaking in tightly capped tubes, 1 h at 37°C.

2- Then proteinase K was added followed by incubation at 37°C/12h.

3- Extract samples with an equal volume of phenol.

4- Add one volume of phenol/chlorophorm/isoamyl alcohol. Centrifuge at 10,000 rpm for 10 minutes (If phases do not resolve well, add another volume digestion buffer and repeat centrifugation. If thick white material appears at interface, repeat organic extraction). Transfer top layer (aqueous) to a new tube.

5- Add 1/10 vol of 5 M NaCl and 1vol of 100% cold ethanol. Centrifuge at 10,000 rpm for 2 minutes.

6- Wash with 70% ethanol, air dry and resuspended in Tris EDTA (TE, 10 mM Tris – HCl, 1 mM EDTA, pH 8.0) buffer at 1mg/ml (Gross-Bellard et al., 1972 and Sambrook et al., 1989).

Gel preparation:

Gel was prepared using 1.8% electrophoretic grade agarose (BRL). The agarose was boiled with tris borate EDTA buffer (1X TBE buffer, 89 mM tris, 89 mM boric acid, 2 mM EDTA, pH 8.3), and then 0.5 microgram / ml ethidium bromide was added to agarose mixture at 40°C. Gel was poured and allowed to solidify at room temperature for 1 h before samples were loaded. The gel was stained with ethidium bromide and detected by UV transilluminator. The gel is photographed with plaroid camera to obtain a permanent record of the band pattern in the gel (Aquadro et al., 1992).

RESULTS

In control mice, the mean frequency of chromosome aberrations per 50 cells was 3.83±1.47. Most of the cells (85.33%) have a diploid chromosome number 2N= 40. Treatment of mice with corn oil alone does not induce any significant effect on the bone marrow chromosomal aberrations as shown in Table (I).

The percentage of aberrant cells increased in a concentration-related manner after atrazine treatment, Fig. (1).

Atrazine-induced both chromosome type aberrations and chromatid-type aberrations at all concentrations tested. Even at lower concentrations (1/64 LD_{50}) abnormal metaphases were observed at 1.39 times higher rate than those in untreated control level. About 17.33% of the examined cells showed one or another type of structural chromosomal anomalies following treatment with 1/32 LD_{50} (56.25 mg of atrazine). This value was increased to 18.33% at 112.5 mg/kg concentration. Chromosomal aberrations were observed in 18.67% of the metaphase of mice treated with 225 mg/kg body weight (1/8 LD_{50}).
Mahmoud, (2008) Studies of Atrazine Effects on DNA in Mice by Chromosomal Aberration Test
The centromeric attenuation, Robertsonian translocation and acentric fragments were the main aberrations noticed with atrazine. Secondary constrictions, acentric fragment, double minutes and gaps were rarely observed, Fig. (2). Attentive assessment of the distribution of gaps revealed that the distal regions of the long chromosomes were more vulnerable to the herbicide. Cells containing more than one aberrations were less frequent in occurrence, Fig. (3).

A slight increase in the mitotic index values in atrazine treated animals was noted at the lowest tested dose, then this value was found to be decreased gradually with increase the dose of the herbicide, though it still remained higher compared to untreated animals.

The frequency of hypoploidy also increased with increasing concentrations of atrazine but statistically significant differences from the controls were observed only at the dose of 225 mg/kg body weight. On the other hand, there was a clear dose dependent increase in the induction of polyploidy, Fig. (4). The frequency of polyploid metaphases in the bone marrow cells of mice was significantly increased at all dose levels compared with controls. In polyploid metaphases, the chromosomes presented as a minute and highly shrunken units which were not easy to identify and even impossible to karyotype, Fig. (5). This type of aberration showed the highest score among other types.

**DNA concentration:**

The amount of DNA in the control group was 38.5µg/ml and 30.8µg/ml in liver and kidney tissues, respectively (Table II). After 24hr of treatment with corn oil, the DNA amount in the both tissues were reduced to 36.8µg/ml and 24µg/ml, respectively. The intensity of DNA in mouse liver increased at low concentration of atrazine, while the high concentration of the herbicide reduced such intensity. The daily administration of atrazine for 5 consecutive days at 1/16 LD₅₀ induced marked decrease of DNA concentration in the liver tissue to 30.9µg/ml; Furthermore, the DNA amount in kidney tissue treated with atrazine decreased gradually till it reached 26.2µg/ml in the high dose. While, DNA level in this tissue was 28.4µg/ml and 32.3µg/ml due to treatment with 112.5 mg/kg body weight (1/16 LD₅₀) for 5 and 10 consecutive days, respectively.

![Graph](image)

**Table (II):** The concentration of DNA in both liver and kidney tissues of mice treated with atrazine.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>Corn oil</th>
<th>28.125 mg/kg b.w.</th>
<th>56.25 mg/kg b.w.</th>
<th>112.5 mg/kg b.w.</th>
<th>225 mg/kg b.w.</th>
<th>112.5 mg/kg b.w. for 5 days</th>
<th>112.5 mg/kg b.w. for 10 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>38.5</td>
<td>36.8</td>
<td>46.5</td>
<td>37.2</td>
<td>41.7</td>
<td>29.3</td>
<td>30.9</td>
<td>34.2</td>
</tr>
<tr>
<td>Kidney</td>
<td>30.8</td>
<td>24</td>
<td>30.9</td>
<td>26.9</td>
<td>26.6</td>
<td>26.2</td>
<td>28.4</td>
<td>32.3</td>
</tr>
</tbody>
</table>
Figure (2): Mitotic chromosomes from bone marrow cells of mice treated with atrazine for 24h.

Rt = Robertsonian translocation  ac f = acentric fragment  b = break
R = ring  s c = secondary constractions  d = deletion
Figure (3): Metaphase spread with multiple aberrations.

Rt = Robertsonian translocations  
iso g = isochromatid gap  
e to e assoc = end to end associations  
d = deletion  
g = gap

Figure (4): Frequency of aneuploid and polyploid cells 24h after treatment with atrazine.
Figure (5): Numerical chromosomal aberration in bone marrow cells of mice treated with different doses of atrazine

- a = hypodiploid metaphase with 37 chromosomes.
- b = hyperdiploid metaphase with 41 chromosomes.
- c = polyploidy.

**DISCUSSION**

Triazine herbicides constitute one of the largest groups of herbicides sold in the United States with estimates ranging from 90 to 121 million ponds of active ingredient used annually (Goldman, 1994). These herbicides are used both for pre-emergence and post-emergence control of annual grasses and broadleaf weeds during cultivation of maize, wheat, sorghum, sugar cane and conifers (Worthing and Walker, 1983).

Atrazine-induced a variety of chromosomal aberrations (CAs) in the bone marrow cells. The dose-dependent increase in the frequency of chromosome aberrations as observed presently, indicate that the herbicide might be available in greater concentrations to the target cells at higher doses. The lowest concentration of atrazine was the only concentration which did not induce a significant increase in structural chromosomal aberrations frequency. The increase in chromosomal aberrations (CAs) frequency might have resulted from damaged DNA structure during the s phase of the preceding cell cycle. In the present study, the amount of the centromeric fusions (Robertsonian translocations) was significantly higher, suggesting a clastogenic effect of atrazine.

On the other hand, differences in the toxicokinetic profile with the route of administration have, therefore influenced its cytotoxic and cytogenetic effects in the present study. Extensive hydrolytic cleavage (detoxification) and poor adsorption through the gastrointestinal tract appear to have rendered the orally administered atrazine less effective as compared to parenteral (i.p. and s.c.) administration where increased bioavailability and primarily oxidative biotransformation (activation) are likely to contribute to its cytotoxic and clastogenic effects. A similar administration route dependent cytogenetic response to fenvalerate has been reported in mice (Pati and Bhunya, 1989). In addition, the elevation of CAs could be attributed to the pharmacokinetic potential of atrazine as stated by Catenacci *et al.* (1993). Since, the atrazine metabolites are eliminated in urine in slightly longer than 24h : 50% of the amount is excreted in the first 8h following the workshift.
There is clear evidence that atrazine produces similar effects on plant and mammalian cells. It induces sister chromatid exchanges (SCEs) in root meristem cells of *Allium cepa* during the S phase, suggesting that the plant cell system shows similarities and may replace mammalian cells in screening and testing mutagens *in vitro*. Therefore, long treatment with maleic hydrazide (MH) will certainly cover the S period, while pulse treatment with MH will presumably damage few cells which are not in the S phase (Gonzalez-Gil and Navarrete, 1986).

Moreover, MH increased dominant lethals, X-linked recessive lethals and X or y loss in *Drosophila melanogaster* (Murnik and Nash, 1977).

As it has been shown by Natarajan *et al.* (1981) that false-positive results in the sister chromatid exchange (SCE) assay may be caused by inhibitors of poly (ADP-ribose) - polymerase.

The possibility that the mutagenic activity of MH was due to the presence of impurities such as hydrazine or other compounds was considered. Therefore, Chinese hamster ovary (CHO) cells were treated with three different samples of MH of varying grades of purity. The magnitude of SCEs induction was similar for the three different samples, suggesting that the effect was not due to the presence of impurities. In addition, maleic hydrazide does not seem to induce SCEs in bone marrow of mice treated *in vivo* (Meschini *et al.*, 1988). These data obtained *in vivo* cannot be considered conclusive, as it is well known that cells other than bone marrow may be sensitive cell population.

Similarly, Kligerman *et al.* (1993) found a concentration related increase in SCE in the splenocytes of rat and mouse subjected to the mixture of pesticides and ammonium nitrate that stimulated groundwater in California.

In 2004, Malik *et al.* suggested that an important part in the variation of SCE frequency reported by different laboratories when conventional SCE analysis is applied after exposure to a certain concentration of atrazine, is due to differences in cell cycle kinetics of cultured lymphocytes, rather than to a true biological variation in the cytogenetic end point used.

Additionally, in chromosome studies, MH itself induced chromosomal aberrations in Chinese hamster V79 cells when directly added to the medium, as previously demonstrated by other workers (Nishi *et al.*, 1979). Similar studies reported by Taets *et al.* (1998) and Rayburn *et al.* (2001) using CHO cells.

In a series of *in vivo* and *in vitro* studies, Meisner *et al.* (1992) report problematic findings, concerning the clastogenicity of atrazine. This study's methodology is open to question because bone marrow, a rapid cycling tissue, should not be used for analyses following chronic treatments as damaged cells are lost during subsequent cell divisions. Nevertheless, the authors report no increase in CAs in mice subjected to atrazine through their drinking water. In the *in vitro* part of this study, the authors treated human lymphocytes with from 0.01 to 1.0 µg/ml atrazine and reported a small increase in CAs at 0.1 and 100 µg/ml.


However, several authors indicated that the herbicide atrazine was not effective using sister-chromatid exchange (SCE), chromosome aberrations and/or micronuclei (MN) in cultured human peripheral blood lymphocytes, irrespective of the presence of metabolic activation, which would mean a general lack of effectiveness of atrazine to induce clastogenic and aneugenic damage in cultured human lymphocytes (Ribas *et al.*, 1998; Kligerman *et al.*, 2000 and Malik *et al.*, 2004). The lower sensitivity of MH on mammals and mammalian cells, as well as bacteria and fungi, may depend on the higher capacity of detoxication or lower levels to break down MH into products acting on cells cytogenetically, rather than that MH can not penetrate and reach the target nuclei better in mammalian cells than in plant cells (Nishi *et al.*, 1979).

Recently, Bouilly *et al.* (2006) described aneuploidy in the Pacific oyster, *Crassostrea gigas*, contaminated by atrazine using the G-banding technique and the restriction enzyme HaeIII.

On the other hand, there was a non significant difference (P>0.05) in mitotic index of treated mice when compared to control group. The present results are in agreement with the findings of Ugulava and Khubultiya (1975) who reported that the herbicides Dacthal (Chlorthal-dimethyl), diphenamid, atrazine, monuron and simazine caused insignificant changes in mitotic activity and chromosome changes were non-specific and not different from spontaneous mutations, nuclear proteins and DNA in onion root cells were affected hardly at all.

In 1992, Meisner *et al.* demonstrated dose related cytogenetic damage not associated with mitotic inhibition or cell death, with damage due to the alachlor-atrazine combination suggesting an additive model in human lymphocytes. Similary, Zeljezic and Garaj-Vrhovac (2002) mentioned that there were no differences in the proliferative rate index (PRI) between the control and treated group regardless of the sampling period in workers occupationally.
exposed to a mixture of pesticides (atrazine, alachlor, cyanazine, 2,4-dichlorophenoxyacetic acid and malathion).

The increase in polyploid cells in treated animals was significant at all dose levels but the increase in hypoploidy was found to be significant only at the higher dose. Polyploidy observed after treatment with atrazine caused an effect on the mitotic spindle. The relative damage of the spindle and the chromosomes may be distributed to the extent that the spindle is aborted at some extent or is absent, which results in the duplication of chromosome complement or polyploidy. This type of polyploidy is known as endopolyploidy and may be caused by different processes (Geitter, 1953 and Temtamy et al., 1982).

Endomitotic reduplication is a specialized process of doubling the chromosome material with the tendency of cells to pass from G2 to the next s phase without undergoing cell division. Microtubular / spindle disturbances are considered important to this process with implications of possible aneuploidy (Dean and Danford, 1984). Moreover, the induction of aneuploidy and polyploidy might be determined by quite different mechanisms, the former occurred at lower doses, involving missegregation of one or a few chromosomes, the latter occurred at higher doses, affecting the whole mitotic apparatus (Athwal and Sandhu, 1985).

On the other hand, induction of endomitotic reduplication has also been observed with a number of chemicals that influence DNA replication (Bean et al., 1992 and Gurr et al., 1993). A variety of chemicals including spindle disturbances also produce endomitotic reduplication and/or aneuploidy (Warr et al., 1993).

From the obtained results, it is clear that the decrease in the DNA content was depending on the tissue examined. The DNA amount was gradually decreased in the kidney tissue following 28,125, 56,25, 112.5 and 225 mg/kg of atrazine exposure for one day. However, no dose response could be found for DNA amount in the liver tissue. Moreover, the results showed that, there was a slight decrease in the concentration of DNA in kidney cells after oral administration of atrazine compared to liver cells of mice. The present results suggest that atrazine may be associated with genetic toxicity and that liver DNA was the target for atrazine.

In 1985, Gluth et al. mentioned that the amount of radioactivity which derived from 14C – labeled pollutants was 3-4 in liver, kidney and intestine, but just 1 in blood, muscle and gills of carp exposed for 6,24 and 72hrs to high external concentrations of atrazine.

Neskovic et al. (1993) found that atrazine led to changes of varying intensity, depending on the parameter tested, the organs and tissues examined, as well as the atrazine concentration in carp. Furthermore, Scutaru et al. (1998) showed that atrazine concentrations measured in the liver of rats were higher than those found in the kidney, but both can be ranked as low compared with the amount of the administered doses.

In addition, the effects of a multiple dose indicated that the DNA amount in mouse liver and kidney were restored to normal levels, presumably due to a rapid degradation and elimination of atrazine. The present results is in agreement with the data obtained by Gordana et al. (1982) who indicated that ceruloplasmin and acid phosphatase activities were returned to normal levels in liver, kidney and spleen of rats administered a second dose of ATR after the recovery from the first treatment.

As the concentration of atrazine increased in the present work, the chromosomal aberrations values gradually increased as well, suggesting a high frequency of lesions in the DNA molecule. Similarly, Timson (1968) expected, theoretically, from the chemical structure of maleic hydrazide (MH) that it can interfere with DNA synthesis by substitution for the pyrimidines, and thus it would lead to faulty replication leading to a reduction in the number of successful mitoses in phytohaemagglutinin-stimulated human lymphocytes.

Some more recent studies have also been contradictory. Apparently, maleic hydrazide does not damage DNA per se, but only after transformation by the plant metabolism to some damaging derivatives (Plew and Gentile, 1982). Pino et al. (1988) found atrazine to cause a very marginal increase in alkaline labile sites using the DNA alkaline elution assay on DNA from the stomach, kidney, and liver of rats treated orally with atrazine.

Studies by Taets et al. (1998) made use of flow cytometry attempt to measure clastogenicity induced by three triazines alone and in various combinations. These authors report that atrazine can induce clastogenicity as measured by changes in the coefficient of various (CV) of the DNA content of the G1 peak at concentrations down to 0.003 µg/ml.

The absence of DNA migration induced by maleic hydrazide was not affected in tobacco by either pH of the MH solution, the sampling time after MH treatment or continuous MH treatment for 14 days (Gichner et al., 2000). Futhermore, using Comet assay, Tennant et al. (2001) showed that atrazine in concentrations of 250 and 500 mg/kg body weight significantly increased DNA migration in leukocytes of mice treated in vivo.

Marc et al. (2004) stated that the extent of the inhibition of DNA synthesis by formulated glyphosate was correlated with the effect on the cell cycle of sea
urchin development. They concluded that formulated glyphosate's effect on the cell cycle is exerted at the level of the DNA response checkpoint of S phase. In 2005, Chang et al. showed dose-related increases in DNA damage in blood cells of common carp (Cyprinus carpio) treated with alachlor or atrazine at environmentally relevant concentrations (<100 ppb) using the single cell gel electrophoresis method. Atrazine at concentrations as low as 7 ppb induced liver cytochrome p450 IAI gene expression.

In 2006, Liu et al. found that atrazine was able to cause apoptosis in grass carp (Ctenopharyngodon idella) cells from cell line ZC7901. Moreover, DNA fragmentation was detected by the TUNEL reaction and agarose gel electrophoresis.

Unlike atrazine, the commercial formulation Gesaprim and the adjuvant mixture increased DNA damage and the number of apoptotic cells in human lymphocytes. Metabolic activation additionally enhanced the DNA damaging potential of Gesaprim and the adjuvant mixture but did not affect atrazine genotoxicity (Zeljezic et al., 2006).

In conclusion, by using the gel agarose DNA electrophoresis on both liver and kidney, genotoxic effects of atrazine in vivo can be detected and more knowledge on the specificity of its mutagenic action for both organs could be gained. Also, used on different organs in in vivo genotoxicity studies, the gel DNA electrophoresis assay could provide a good assessment of potential pesticide carcinogenicity.

Finally, the results of the present study indicate that atrazine have definite interactions with DNA metabolism in mice, resulting in chromosomal aberrations, indicating potential mutagenic effects. Human exposure to this agent should be restricted.

REFERENCES


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