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Clastogenic Effect of Tetrahydrofuran (THF): a Comparative Study on Five Rodent Species

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Abstract: The possible clastogenic activity of tetrahydrofuran (THF), a synthetic organic substance, was explored employing two cytogenetic endpoints, i.e., chromosome aberrations (CA) and mitotic index (MI) analyses *in vivo*. The assays were carried out with ICR line laboratory mice as well as some widespread small mammal species in Bulgaria: yellow-necked wood mouse *Apodemus flavicollis*, mound-building mouse *Mus spicilegus*, bank voles *Clethrionomys glareolus* (= *Myodes glareolus*) and common vole *Microtus arvalis*. THF was introduced at 3.8 mg/kg b. w. (body weight) (0.01 mg/mL) by a single intraperitoneal (ip) administration. Cells with various structural CA were noted with significant ($p < 0.01$) differences in the frequencies of CA and MI between THF-treated groups and the negative control groups at dose or time interval used. The results of the present study reflect a positive *in vivo* clastogenic potential of THF.

Key words: Tetrahydrofuran, genotoxicity, chromosome aberration analysis, mitotic index, genetic chromosome instability

Introduction

Tetrahydrofuran (THF) is a synthetic organic substance that does not occur naturally in the environment (ACGIH 2001). It is used as a versatile solvent in the production of adhesives, varnishes, printing inks, fats, oils, unvulcanised rubber, etc., in the manufacturing of items for packaging, transportation and storage of food (NTP 1998, Dannan 2015) as well as in chemical and pharmaceutical syntheses (Parod 2014, Yin et al. 2017). This solvent has become a common contaminant in aquatic environments and groundwaters near industrial areas where the compound is used (Isaacson et al. 2006, Yao et al. 2012, Dannan 2015). When used as a solvent, significant amounts of THF can be released into environment due to its high vapour pressure and water solubility (Yao et al. 2012). In case of unintentional release, it can lead to partial volatilisation in the atmosphere (Fowles et al. 2013). Occupational exposure to THF occurs through inhalation or dermal contact with solvents and adhesives (Dannan 2015). Yao et al. (2010) suggested that THF is much more toxic than previously reported, indicating its acute toxicity to microorganisms. Once released into the environment, the behaviour of THF is not entirely known and scarce monitoring data are available (NTP 1998, Afghan et al. 2019).

Little is known about the cellular and toxicokinetic toxicity of THF (Moody 1991, Chhabra et al. 1998) or whether THF metabolises into more toxic substances (NTP 1998). Earlier studies (Kawata & Ito 1984, Hard & Seely 2005) indicated kidney as one of the target organs affected by THF toxicity. THF was

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not mutagenic in *Salmonella* micronucleus test and did not induce sister chromatid exchanges or chromosomal aberrations in ovarian cells (Gamer et al. 2002). According to the NTP (1998), in male B6C3F1 mice, after intraperitoneal injection of THF at doses up to 2000 mg/kg, a significant increase in the number of chromosomal aberrations in the bone marrow cells was not observed. However, periodic American Conference of Government Industrial Hygienists (ACGIH 2001) confirmed that THF is a carcinogen in animals with unknown relevance to humans.

According to the guidelines for carcinogenic risk assessment (U.S. EPA 2005), there is suggestive evidence of carcinogenic potential after inhalation of THF in humans. A number of evidence indicates that THF is carcinogenic for more than one species, gender and place of tumorigenesis and, therefore, these data are indicative of a carcinogenic potential in humans after exposure to THF (U.S. EPA 2005, 2012). The active ingredient of THF is released during the incineration of organic waste and heat treatment of food. Furan is toxic and a proven carcinogen in laboratory animals (Hermida et al. 2006, von Tungeln et al. 2017). The risk assessment of furan requires determining the role of genotoxicity as a causative factor for its carcinogenicity. Ding et al. (2012) also described DNA damage in the liver of rats treated with a cancer bioassay dose of furan. According to Hibi et al. (2017), genotoxicity mechanisms are not responsible for the initial stages of furan induced hepatocarcinogenesis but cell proliferation. Moreover, nongenotoxic mechanism of furan action was suggested by de Conti et al. (2016).

As regards to human risk assessment, one study about the genotoxic potential in humans (Funes-Cravioto et al. 1977) reported increased levels of chromosomal aberrations in persons exposed to THF as compared to control groups. Overall, data presented about the toxic, clastogenic and carcinogenic effect of THF are incomplete and contradictory, which justifies further research about the biological action of this organic pollutant (e.g. Afghan et al. 2019, Chhabra et al. 1998, Gamer et al. 2002, Fenner-Crisp et al. 2011).

Studies that use animal test systems and answers they could give (Topashka-Ancheva & Gerasimova 2012) may be the connection between observations based on animals with those involving humans (O'Brien et al. 1993). The study of cytogenetic alterations is considered extremely important in the human context (International Commission for Protection Against Environmental Mutagens and Carcinogens 1983, WHO 1985). Rodents frequently serve as models to evaluate human risk in ecotoxicological studies (Shore & Rattner 2001). The use of small mammals to assess human exposure to toxic environmental contaminants is widely accepted and reliable (O'Brien et al. 1993).

In studies of environmental genotoxicity, some authors suggest performing comparative studies on species occupying similar or overlapping ecological niches. Such studies are necessary for accurately evaluating environmental quality, identifying sensitive species for biomonitoring as well as for assessing interspecies sensitivity to contaminants (Wren 1986, Abramsson-Zetterberg et al. 1997, Meier et al. 1999, de Souza Bueno et al. 2000, Ieradi et al. 2003, Sánchez-Chardi et al. 2007).

Limited data are available on changes in chromosomal structure in wild small mammals after acute treatment by a particular genotoxin under strictly controlled laboratory conditions and compared to laboratory mice. The hypothesis is that the chromosomes forming the karyotypes of various species of small mammals react in a specific way to the action of the same genotoxin. Based on this data consideration, we formulated the objective of the present work – evaluation of the potential clastogenic and cytotoxic effect of THF through classical cytogenetic methods. The study utilised laboratory animals and various wild small rodent species, some of which are relatively widespread in Bulgaria. The main objective was to compare the results obtained from different species and justify the need to include other species as test models, aside from those already commonly used in laboratory practice. The conclusions would give a more reliable assessment of the environmental pollutants' impact on the gene pool of populations, including humans.

Materials and Methods

Experimental animals

In the experiments, we used laboratory ICR mice as well as some widespread small mammal species in Bulgaria: *Apodemus flavicollis* (Melchior, 1834) and *Mus spicilegus* (Petényi, 1882) of the family Muridae as well as *Clethrionomys glareolus* (Schreber, 1780) (=*Myodes glareolus*) and *Microtus arvalis* (Pallas, 1778) of the family Cricetidae (order Rodentia). Model species have been selected to meet the following conditions (Topashka-Ancheva & Gerasimova 2012): they are widespread and are situated low in the food chain, have short reproductive cycle and produce several generations per year (Metcheva et al. 2001).

All wild species of small mammals were caught in spring – late autumn using live traps (Sherman type) in several conditionally unpolluted

regions in Bulgaria. The sampling spots were: SW Rila Mountains, near the Parangalitsa Field Station of IBER–BAS (*C. glareolus* and *A. flavicollis*); Vitosha Nature Park, west of the Dragalevtsi aerial lift (*C. glareolus* and *A. flavicollis*); the Pleven Region – agroecosystem in the south-western part of Knezha Town (*M. spicilegus* and *M. arvalis*). For these areas, there are no data on natural or anthropogenic heavy metals and organochlorine loads and they are considered as conditionally unpolluted regions.

Differentiation of *A. flavicollis* from *A. sylvaticus* was performed by amplification of a region of cytochrome B from the mitochondrial genome approximately 300 bp in length, following Michaux et al. (2001) and Gerasimova (2012).

Previous authors (e.g. Metcheva et al. 2001, Martiniaková et al. 2010) have identified *C. glareolus* and *A. flavicollis* as suitable species for environmental pollution assessment. Martiniaková et al. (2010) considered *C. glareolus* as a more sensitive species to heavy metal contamination as compared to *A. flavicollis*. *Clethrionomys glareolus* is also a common biomarker in Europe due to its widespread distribution in forest ecosystems and high sensitivity to contaminants (Bujalska & Hansson 2000, Smolich et al. 2011). *Mus spicilegus* has been selected because, along with voles, is one of the most common species in the agroecosystems of northern and north-eastern Bulgaria (Popov et al. 2004); it is also highly exposed to the negative influence of a number of potentially mutagenic substances in its habitat. In Bulgaria, the mound-building mice (*M. spicilegus*) construct their overwintering structures back in September (Tognetti et al. 2017). The live traps were distributed around or on the mound in late autumn to ensure capturing only the targeted species.

Experimental conditions

The experiments were done only with adult mature individuals. The data for the species and the number of male and female individuals used in the experiments are presented in Table 1. Experimental treatments were performed not earlier than 72 hours after capturing of the wild model species.

Laboratory white mice ICR (2n=40) (♂♀=18), weighting 20 ± 1.5 g, were delivered from the Slivnitza Animal Breeding House of the Bulgarian Academy of Sciences. Animals were kept at standard conditions at temperature 20–22ºC, photoperiod 7 am to 7 pm, with free access to standard animal food for laboratory animals "Rodents" (Vitaprot Ltd., Kostinbrod, Bulgaria, prescription 456-1-12) and to water. The experiments were conducted according to approved protocols and in compliance with the requirements of the European Convention for Protection of Vertebrate Animals used for experimental and other Specific Purposes and the current Bulgarian laws and regulations.

Animals were divided into three groups: experimental, negative and positive control groups. THF $(CH_2)4O$ (3.8 mg/kg body weight) (Merck, Germany), dissolved in 0.9% NaCl solution, was injected intraperitoneally (ip) only once into each experimental animal (n=53, 27♂ and 26♀). The highest dose (3.8 mg/kg b.w. THF), at which 100% survival of experimental animals was observed, had been determined after preliminary experiments. Intraperitoneal administration is commonly used due to its simplicity and as it ensures that the bone marrow is adequately exposed to the substance (Preston et al. 1987). Negative control groups (n=44, 24♂ and 20♀) received only 0.9% NaCl solution. The positive control group (n=55, 30♂ and 25♀) received Mitomycin C (MMC) (3.5 mg/kg) (Sigma EC No 200-008-6), a clastogenic agent with proven genotoxic effect and high incidence of chromosomal aberrations induction (OECD 1997).

Cytogenetic endpoints

The cytogenetical analyses were mainly performed according to the protocol described by Preston et al. (1987). One hour before bone marrow cell isolation a mitotic inhibitor colchicine, 0.04 mg /g b.w., was injected. Animals were euthanised with diethyl ether; bone marrow cells were flushed from femur and hypnotised in a 0.075 M potassium chloride at 37ºC for 15 min. Thereafter the cells were fixed in cold methanol: glacial acetic acid (3:1), resuspended and dropped on precleaned cold wet slides and air dried. The slides were stained in 5 % Giemsa solution (Sigma Diagnostic). Up to 50 well-scattered metaphase plates were analysed from each animal using light microscopy (Cetopan Reichert, Austria) x 1000.

The main types of aberrations – breaks, fragments, exchanges (centromere / centromeric fusions, telomere/telomeric fusions) and pericentric inversions were scored separately.

Statistical analyses

The frequencies of chromosomal aberrations (in percentage) were determined for each animal and up to 50 metaphases (number of metaphases with aberrations / 50 * 100) were scored. The results for each experimental group are presented as mean ± SD. The median of the samples was compared for statistically significant differences using a two-tailed nonparametric test for small samples comparison – Mann-Whitney U-test calculator for inappropriate samples

(http://www.holah.karoo.net/Mann-Whitney%20U-test.xls). The interpretation was carried out by the instructions of Fowler et al. (1998). Statistical significance was set at $p < 0.05$. Since the percentage of chromosomal aberrations among males and females was not found to be statistically significant, the results for both sexes were combined.

The indicator for cytotoxicity, the mitotic index (MI) in per mil (‰), was also reported by counting all dividing bone marrow cells among at least 1500 accounted cells for each model animal (statmokinetic method) (Darzynkiewicz 1987). The formula is MI = A / B x 1000, where A is the number of dividing cells and B is the number of counted cells.

Results

Clastogenic effect of tetrahydrofuran (THF)

The possible genotoxic potential of THF was evaluated using a conventional chromosomal aberration test. Data from the cytogenetic analysis performed on 3.8 mg / kg THF-treated bone marrow cells are presented in Table 1.

Chromatid-type aberrations (breaks and fragments) predominated in the experimental groups of all THF-treated rodent species (Figure 1). Chromosomal exchanges (c/c and t/t fusions) were characteristic mainly for ICR mice and *M. spicilegus* because of their acrocentric type of chromosomes. The highest percentage of breaks and fragments from all aberrations obtained were observed in *M. arvalis* (96 %) and *A. flavicollis* (95 %), while the lowest were observed in the group of ICR mice (57.6 %). The results showed the highest percentage of cells with aberrant chromosomes in the laboratory line ICR mice (14.75% ± 2.60). A significant difference in the percentage of damaged bone marrow cells after experimental treatment with THF was reported among laboratory mice line and all the other experimental groups of wild rodents: ICR / *M. spicilegus* ($U = 0$, $p < 0.01$), ICR / *M. arvalis* ($U = 0$, $p < 0.01$), ICR / *C. glareolus* ($U = 0$, $p < 0.01$) and ICR / *A. flavicollis* ($U = 0$, $p < 0.01$) (Table 1).

The bone marrow cells of the other wild rodent species studied have shown a high similarity concerning the response to the genotoxin applied. Data

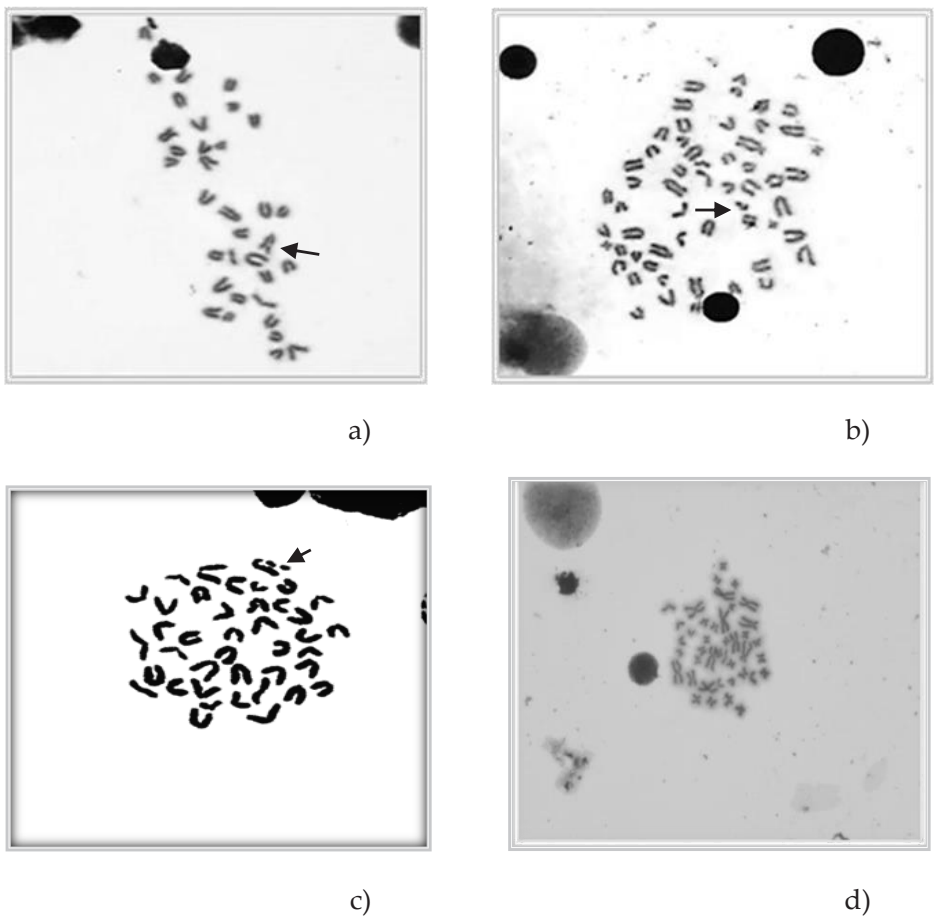

Fig 1. Metaphase of ICR laboratory mouse with a break (a); and male *C. glareolus* with a fragment (b); double break in *M. spicilegus* after single ip supplementation with THF (3.8 mg/kg b.w.) (c); normal metaphase plate of *M. arvalis* (d).

Table 1. Number and frequency of chromosomal aberrations, reported in laboratory mice ICR line, *M. spicilegus*, *M. arvalis*, *C. glareolus* and *A. flavicollis* after THF ip supplementation (3.8 mg/kg b.w./24 h). * p <0.05, ** p <0.01; **a** – compared to control; **b** – compared to MMC. Aberrations: c/c – centromere/centromeric fusion; t/t – telomere/telomeric fusion; c/t – centromere/telomeric fusion.

Tested species and substances	Metaphases analysed	Types of aberrations						Cells with chromosomal aberrations % (mean±SD)	Statistical significance	
		Breaks	Fragments	Cells with exchanges			Pericentric inversions		a	b
				c/c	t/t	c/t				
ICR control	5♂ 5♀ 250	3	0	2	0	0	0	1.0 ± 1.05		
ICR THF	4♂ 4♀ 400	19	15	23	2	0	0	14.75 ± 2.60	* *	* *
ICR MMC	5♂ 5♀ 500	57	75	17	2	0	6	31.40 ± 2.69		
M. spicilegus / control	8♂ 2♀ 500	3	10	5	0	0	0	3.60 ± 1.26		
M. spicilegus / THF	4♂ 8♀ 600	20	19	6	0	0	0	7.5 ± 1.51	* *	* *
M. spicilegus / MMC	6♂ 4♀ 500	49	50	5	2	0	2	21.6 ± 4.60		
M. arvalis / control	3♂ 3♀ 500	4	4	0	0	0	0	2.67 ± 1.03		
M. arvalis / THF	4♂ 5♀ 384	20	5	0	1	0	0	6.93 ± 1.56	* *	*
M. arvalis / MMC	9♂ 8♀ 812	58	29	0	0	0	4	10.80 ± 1.25		
C. glareolus / control	5♂ 5♀ 471	3	2	1	0	0	0	1.2 ± 1.40		
C. glareolus / THF	4♂ 6♀ 500	19	12	3	3	0	0	7.4 ± 1.35	* *	*
C. glareolus / MMC	5♂ 3♀ 365	23	13	1	2	0	1	10.69 ± 2.22		
A. flavicollis / control	3♂ 5♀ 498	4	3	2	0	0	2	2.25 ± 1.28		
A. flavicollis / THF	11♂ 3♀ 621	19	23	2	0	0	0	6.85 ± 1.47	* *	*
A. flavicollis / MMC	5♂ 5♀ 500	33	45	10	4	0	12	11.01 ± 3.44		

on absolute values for the percentage of cells with aberrations in *M. spicilegus*, *M. arvalis*, *C. glareolus* and *A. flavicollis* did not differ significantly ($p \geq 0.05$; Table 1). All treated groups showed a significantly higher percentage of damaged cells compared with their corresponding untreated controls (ICR – $U = 0$, $p < 0.01$; *M. spicilegus* – $U = 2.5$, $p < 0.01$; *M. arvalis* – $U = 1$, $p < 0.01$; *C. glareolus* – $U = 0$, $p < 0.01$; *A. flavicollis* – $U = 1$, $p < 0.01$; Table 1). The comparison between the experimental groups of wild small mammals showed no statistically significant differences among median values of the data set: *M. spicilegus* / *M. arvalis* $U = 50$, $p > 0.05$; *M. spicilegus* / *C. glareolus* $U = 58.5$, $p > 0.05$; *M. spicilegus* / *A. flavicollis* $U = 66$, $p > 0.05$; *M. arvalis* / *C. glareolus* $U = 43$, $p > 0.05$; *M. arvalis* / *A. flavicollis* $U = 54.5$, $p > 0.05$; *C. glareolus* / *A. flavicollis* – $U = 55.5$ $p > 0.05$.

In all MMC treated experimental groups (3.5 mg / kg), significant changes in the structure of the bone marrow cells' chromosomes - breaks, fragments and centromere-centromeric fusions were observed. Breaks and fragments significantly predominated over chromosomal type of aberrations (from 75% in *A. flavicollis*, 84% of all observed chromosomal rearrangements in laboratory mice, 90% in *C. glareolus*, 91.7% in *M. spicilegus* up to 96 % in *M. arvalis*). The distribution of aberration types obtained in our experiments confirmed the specific chromosomal damage after treatment with the alkylating agent MMC.

The experimental groups of all small mammalian species, treated with MMC, showed a higher percentage of damaged cells compared to their respective untreated controls and there was a statistically significant difference between median values ($U = 0$, $p < 0.01$). There was also a statistically significant difference between all experimental groups treated with THF and the groups of the respective species injected with MMC ($p < 0.05$; $p < 0.01$). The

Table 2. Mitotic index reported in laboratory mice ICR line, *M. spicilegus*, *M. arvalis*, *C. glareolus* and *A. flavicollis* after THF ip supplementation (3.8 mg/kg b.w./24 h). * p <0.05, ** p <0.01; **a** – compared to control; **b** – compared to MMC.

Tested species and substance	Number of individuals analysed	Metaphases analysed	Mitotic Index ‰ (mean ± SD)	Statistical significance	
				a	b
ICR control	5♂ 5♀ 250	15 129	10.71 ± 3.15		
ICR THF	4♂ 4♀ 400	12 245	6.56 ± 1.63	* *	*
ICR MMC	5♂ 5♀ 500	6 112	5.73 ± 2.57		
M. spicilegus / control	8♂ 2♀ 500	15 170	10.23 ± 5.44		
M. spicilegus / THF	4♂ 8♀ 600	18 099	6.96 ± 1.66		*
M. spicilegus / MMC	6♂ 4♀ 500	13 596	7.74 ± 2.05		
M. arvalis / control	3♂ 3♀ 500	9 054	9.18 ± 1.54		
M. arvalis / THF	4♂ 5♀ 384	29 008	4.03 ± 2.91	* *	* *
M. arvalis / MMC	9♂ 8♀ 812	6 095	4.32 ± 1.57		
C. glareolus / control	5♂ 5♀ 471	15 057	9.96 ± 2.26		
C. glareolus / THF	4♂ 6♀ 500	15 180	8.17 ± 1.88		* *
C. glareolus / MMC	5♂ 3♀ 365	12 096	6.28 ± 1.80		
A. flavicollis / control	3♂ 5♀ 498	12 037	8.98 ± 3.79		
A. flavicollis / THF	11♂ 3♀ 621	22 643	6.15 ± 1.90		* *
A. flavicollis / MMC	5♂ 5♀ 500	15 190	6.86 ± 2.87		

alkylating agent MMC at the concentration applied significantly damaged the chromosomal structures of all small mammal species compared to THF.

From these results, it was seen that the laboratory ICR line exhibited three-fold higher chromosome sensitivity compared to the treated with MMC experimental groups of *C. glareolus*, *M. arvalis* and A. *flavicollis*. Laboratory mice also showed a significantly higher sensitivity to the chromosome-damaging effect of MMC compared to the closely related species *M. spicilegus*. The results unequivocally confirm the clastogenic effect of MMC and, in addition, the data suggest that the laboratory mouse is a more sensitive species to this type of damaging agent. The values of chromosomal aberrations obtained after treatment with THF were significantly lower compared to those values obtained after supplementation with MMC. These results suggest a moderate clastogenic effect of THF.

Antiproliferative effect of THF

The data about the proliferative activity in all studied bone marrow cell populations are presented in Table 2. It is noticeable that in the control groups of all studied species, the reported values of the mitotic index are approximately the same with minor variations – from 10.23 ‰ ± 5.44 in *M. spicilegus* to 8.98 ‰ ± 3.79 in *A. flavicollis*. There is no significant difference between the medians of the control values obtained between the five small mammal species in the THF experiment (p≥0.05). When comparing the proliferative activity among the small mammal species studied with their untreated control groups, a significant suppression of mitotic activity was found in the experimental groups of ICR mice (U = 8; p < 0.01) and *M. arvalis* (U = 4; p < 0.01) (Table 2). Such a difference between treated experimental groups and their respective untreated controls was not reported in *M. spicilegus* (U = 41.5; p > 0.05), *C. glareolus* (U = 28; p > 0.05) and *A. flavicollis* (U = 28.5; p > 0.05) (p≥0.05). These results indicated that inhibition of the proliferative activity should not be taken as a characteristic feature of the THF action.

From the analysis of the results, it is obvious that the values of the mitotic index in all treated groups varied slightly. There was statistically significant difference among medians in the groups of *M. spicilegus* and *M. arvalis* treated with THF (U = 25.5, p <0.05); *M. arvalis* and *C. glareolus* (U = 14.5, p <0.01), as well as between *C. glareolus* and *A. flavicollis* (U = 31, p <0.05). Among all the other experimental groups treated with THF, reliable differences among medians were not calculated (ICR / *M. spicilegus* U = 36.5, p > 0.05; ICR / *M. arvalis* U = 22, p > 0.05; ICR / *C. glareolus* U = 19, p > 0.05; ICR / *A. flavicollis* U = 53.5, p > 0.05; *M. spicilegus*/ *C. glareolus* U = 36.5, p > 0.05; *M. spicilegus* / *A. flavicollis* U = 63, p > 0.05; *M. arvalis* / *A. flavicollis* U = 38, p > 0.05). The values of the mitotic index in all MMC-treated groups also varied slightly (Table 2). Regarding the antiproliferative activity of MMC in the bone marrow cells of different species, it was

found that the cell division in all species was strongly suppressed compared to their untreated controls (ICR/ control U = 4, $p < 0.05$, *M. spicilegus* / control U = 12, $p < 0.05$; *M. arvalis* / control U = 0, $p < 0.01$; *C. glareolus* / control U = 6, $p < 0.01$; *A. flavicollis* / control U = 25, $p < 0.01$). A characteristic feature of the MMC action is the delay of mitosis (Dulout & Olivero 1984).

Discussion

Although THF is an established carcinogen based on long-term rodent tests (Chhabra et al. 1998, Gamer et al. 2002), several cytogenetic studies provide conflicting or negative results for the clastogenic effect (Galloway et al. 1987, MacGregor et al. 1990, Gamer et al. 2002, Fenner-Crisp et al. 2011, Fowles et al. 2013). Lewis et al. (1996) identified THF as a potential mutagen using the Ames mutagenicity test for Salmonella, which did not show a positive effect in the mammalian micronucleus test.

Many results from genotoxicity studies of THF have been presented by NTP (1998), with very few data proving the genetic toxicity of THF in *in vivo* tests. NTP (1998) concluded that there was insufficient evidence of genotoxic activity. Gibson et al. (1997) reported that THF treatment did not result in the formation of an increased percentage of micronuclei. The study was performed with samples of Syrian hamster embryonic cells placed in high concentrations of THF, which significantly reduced the number of surviving cells in culture.

THF was also evaluated as an inactive agent in an *in vitro* assay with standard BALB/ c-3T3 cell lines (Matthews et al. 1993) and *in vivo* assays in male rats and *Drosophila melanogaster* (Mirsalis et al. 1985, Valencia et al. 1985). There was also no significant increase in the number of chromosomal aberrations per cell or the percentage of bone marrow cells with aberrations (McFee et al. 1992, NTP 1998) after intraperitoneal treatment of male B6C3F1 mice at doses of 500, 1000 to 2000 mg/ kg. However, the same dose (2000 mg/kg) was reported to increase mortality in rats receiving THF orally dissolved in distilled water (Stasenkova & Kochetkova 1963). An *in vivo* Comet and micronucleus assays results detected that furan exposure by gavage on four consecutive days did not induce DNA damage in bone marrow of male F344 rats at any dose tested (Ding et al. 2012).

On the other hand, there are literature data indicating that THF has a genotoxic effect. THF has shown a positive result for chromosomal aberrations and a negative result for sister chromatid exchanges in ovarian cells from the Chinese hamster (NTP 1984). Galloway et al. (1987) reported a slight increase in the total number of chromosomal aberrations in cultured Chinese hamster ovary cells. The predominant types of aberrations have been classified by these authors as breaks and terminal deletions, but they do not consider them to be completely sufficient to declare a positive result in their genotoxicity study.

Of particular interest are the results obtained in a 14-day toxicology study conducted by MacGregor et al. (1990). The authors found that the results for female mice were negative, while in male individuals a slight increase in the frequency of normochromatic erythrocytes with micronuclei was observed. The authors concluded that this test provided a contradictory response to the genotoxic effect of THF.

Clear evidence of THF carcinogenic activity was described by Chhabra et al. (1998), NTP (1998), ACGIH (2001), Gamer et al. (2002) and summarised in Fowles et al. (2013). THF is a weak, non-genotoxic liver carcinogen in mice (Choi et al. 2017, Dannan 2015). Regarding liver tumours described in laboratory mice, some mechanistic data suggest that THF can induce cell proliferation and lead to cell growth promotion (Kawata & Ito 1984). The carcinogenic effect of THF in its long-term effects on various types of experimental mammalian species is very likely to be the final outcome for the genotoxic effects identified in our study after a single THF ip administration.

Concerning the antiproliferative effect of THF, the direct comparison of the data obtained with those from the literature is complicated due to the lack of a sufficient number of targeted studies considering the THF influence to the proliferation of normal cells. Most often these are studies that look for a correlation between cell proliferation and the possible carcinogenic effect of THF. In this respect, the results are contradictory. In a series of short-term studies, the cytotoxic potential of THF was evaluated *in vitro*. The results of these studies show that THF is not cytotoxic (Curvall et al. 1984, Dierickx 1989, Matthews et al. 1993, Dannan 2015). Kerckaert et al. (1996) also did not observe cytotoxicity even at the highest tested THF concentration on Syrian hamster embryonic cells. Matthews et al. (1993) noted that *in vitro* cytotoxicity does not correlate with the carcinogenic activity *in vivo*. Fenner-Crisp et al. (2011) and the U.S. EPA (2012) concluded that THF was neither genotoxic nor cytotoxic.

According to data provided by BASF (2001), exposure to THF does not significantly affect the

mitotic index. The observed increase in the mitotic index in B6C3F1 mice exposed to the highest of the concentrations studied has been explained by the abnormally small number of cells in mitosis identified in the control animals.

In contrast to these studies, Gamer et al. (2002) observed increased cell proliferation in liver cells with high doses of THF by inhalation and suggested that the reported number of cells in mitosis confirmed the data on the S-phase response to the toxic effects of THF. Our data regarding the reliable proliferative activity suppression in the experimental groups of ICR mice and *M. arvalis* were somewhat consistent with the studies of Gamer et al. (2002), which indicated that THF induced dose-dependent apoptosis in renal tissue of male F344 rats and female B6C3F1 mice. BASF (2001) also reported an increase in the number of cells in apoptosis to 168% of that in control at the highest of the test concentrations.

In regard to the antiproliferative effect of THF, the lack of statistical difference between the treated experimental groups and their respective untreated controls, reported in *M. spicilegus*, *C. glareolus* and *A. flavicollis*, is further evidence obtained in the present study that there are differences between species in terms of THF metabolism, which evidence is presented by DuPont Haskell Laboratory (2000). According to that study, *in vitro* evidence suggests that there are differences between species in terms of THF metabolism: mice of *Mus musculus* are more sensitive than rats (*Rattus norvegicus*) to some of the effects of THF in a subchronic study (NTP 1998). Differences in THF metabolism have been also found between male and female rodents (induction of renal tumours in male rats and in the liver in female mice (NTP 1998). For genetic monitoring purposes, these facts are particularly important as they imply greater caution when extrapolating data obtained on the biological effects of THF (or any other compound) from studies in laboratory animal species directly on humans.

The results obtained in the present study provide evidence that THF exhibits a clastogenic effect *in vivo* when administered intraperitoneally to five different species of small mammals and confirm some previous data for a certain increase in the frequency of sister chromatid exchanges, chromosomal aberrations and the formation of micronuclei in peripheral blood after intraperitoneal and inhalation treatment with this genotoxin (NTP 1998, Galloway et al. 1987, MacGregor et al. 1990).

The reported high percentage of breaks and fragments in our study can be considered as a reliable indicator of the clastogenicity induced by THF (significant damaging effect on the chromosomes). In the ICR laboratory mice group, treated with THF, a significant percentage of metaphases with chromosomal exchanges, centromeric/centromeric and telomeric/telomeric fusions were also reported.

The data analysis clearly showed that the animals of the genus *Mus* were the most sensitive to the genotoxin applied. Their karyotype sensitivity can be explained by the peculiarities in the chromatin organisation, which builds the chromosomes of species within the genus and may be related to the fact that this genus is phylogenetically relatively young (She et al. 1090, Guenet & Bonhomme 2003, Macholán et al. 2007).

Conclusions

As a result of the genotoxic potential assessment of the widely used in industry organic solvent THF, with the help of a conventional chromosomal aberration test, reliable evidence of clastogenic effect *in vivo* was obtained. We recorded a high percentage of chromatid-type chromosomal rearrangements reported in all small mammals used. This proven genotoxic effect well corresponds to the carcinogenic effect of THF clearly established and accepted in the scientific literature with a prolonged exposure to the organic solvent on various mammalian species in laboratory conditions.

The chromosomal aberration values obtained after treatment with THF were significantly lower compared to the positive MMC control values, suggesting a moderate clastogenic effect of THF. A significant difference in the percentage of damaged bone marrow cells after experimental THF supplementation was reported only in ICR mice and all other groups of wild mammals. The existence of such a difference between the laboratory mouse and the other muroid rodent species supports the thesis of DuPont Haskell Laboratory (2000) and the National Toxicology Program (NTP 1998) that there are interspecific THF metabolism differences. THF also did not manifest antiproliferative activity at the applied concentration. Antiproliferative effect of THF was established only in laboratory mice and *M. arvalis* compared to the untreated control.

The highest response to THF was shown in the ICR laboratory mice, followed by the related species *M. spicilegus*. The highly pronounced karyotype THF reaction of *M. spicilegus* makes it suitable for genotoxic monitoring in those agroecosystems where the species is widespread. The two vole species, *M. arvalis* and *C. glareolus*, showed similar

karyotype sensitivity to the action of THF. The karyotype of *A. flavicollis* reacted similarly to the action of THF compared to *M. spicilegus*, *M. arvalis* and C. *glareolus*. All these results lead to the conclusion that the chromosomal structure of the species of genus *Mus* is more susceptible to the influence of clastogenic factors of different nature.

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