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X-Rays, microwaves and vinyl chloride monomer: their clastogenic and aneugenic activity, using the micronucleus assay on human lymphocytes

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Summary

Chromosome aberration assays, sister-chromatid exchange techniques and micronucleus assays are commonly used methods for biomonitoring genetic material damaged by chemical or physical agents. On the other hand, their aneugenic activity, which can lead to hypoploidy and may also be associated with carcinogenesis, has not been thoroughly investigated. In our study we chose the micronucleus assay with a new mathematical approach to separate clastogenic from aneugenic activity of three well-known mutagens (vinyl chloride monomer, X-rays and microwaves) on the genome of human somatic cells. The comparison of frequencies of size distribution of micronuclei in the lymphocytes of humans exposed to each of these three mutagens showed that X-rays and microwaves were preferentially clastogens while vinyl chloride monomer showed aneugenic activity as well. Microwaves possess some mutagenic characteristics typical of chemical mutagens.

Micronuclei of mammalian cells are formed from the condensation of lagging acentric chromosomes, chromatid fragments or entire chromosomes. Micronuclei which originate from chromatid fragments generally ought to be smaller than those resulting from whole chromosomes (Yamamoto and Kikuchi, 1980; Vanderkerken et al., 1989). X-Ray experiments on mouse bone marrow chromosomes show micronuclei negative for kinetochores (Gudi et al., 1990) which is con-

sistent with the fact that X-rays predominantly induce chromosome breaks (Gudi et al., 1990; Heddle and Carrano, 1977; Eastmond and Tucker, 1989; Fenech and Morley, 1989). Microwave radiation and vinyl chloride monomer have never been analysed from such a viewpoint.

In order to distinguish clastogenic from aneugenic activity of mutagens, we used the micronucleus test as a novel approach in mutagenetic monitoring. We analysed cytochalasin-B cytokinesis-blocked human lymphocytes from populations that had been occupationally exposed to three well-known mutagenic agents: vinyl chloride (Fučić et al., 1990; Purchase et al., 1978; Uzych, 1988), ionizing (Bender et al., 1974; Natarajan et

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al., 1986; Ramalho et al., 1988) and microwave radiation (Sagripanti and Swicord, 1986; Savitz and Calle, 1978; Garaj-Vrhovac and Fučić, 1990). For each agent 100 micronuclei were drawn by camera lucida. Their surface areas were measured on a Morphomat 10 (Opton).

Material and methods

The micronucleus assay was conducted on the lymphocytes of three groups of examinees who had been occupationally exposed to X-rays, microwaves and vinyl chloride monomer. Those ex-

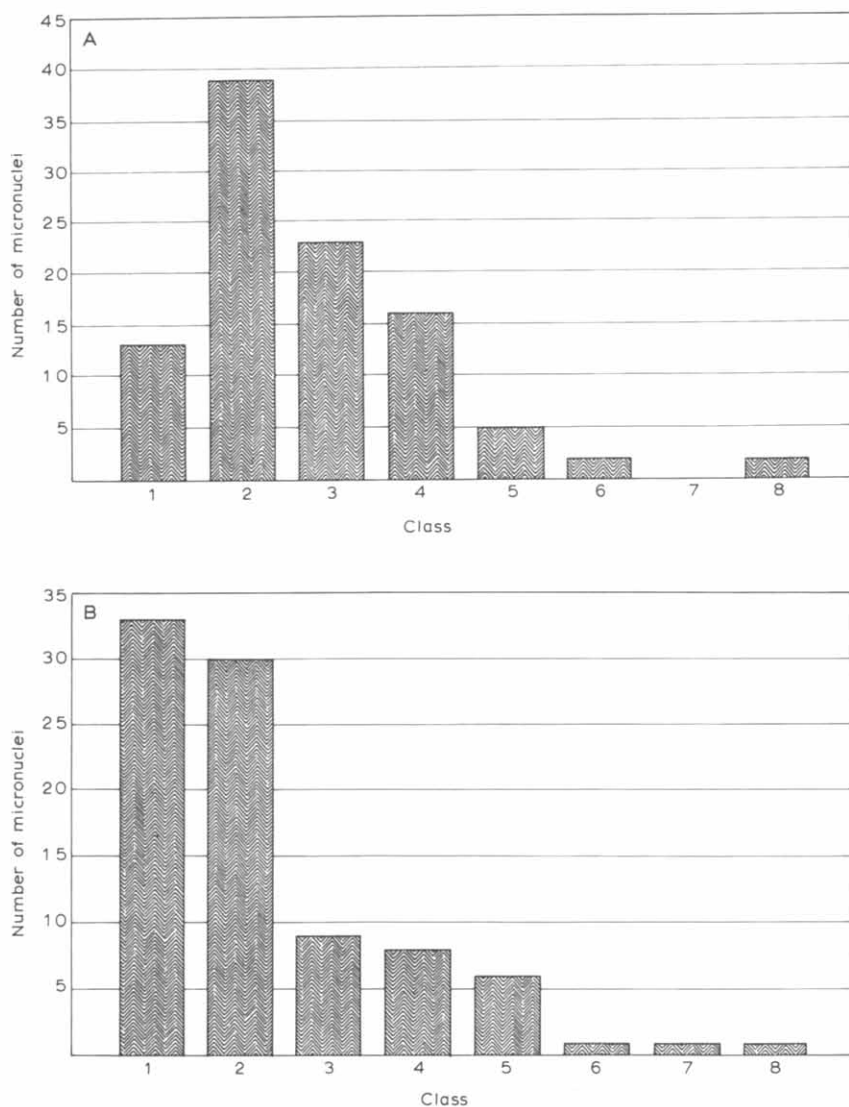


Fig. 1. Distribution of sizes of micronuclei exposed as their surface areas split up into 8 classes. (a) X-Rays, (b) microwaves, (c) vinyl chloride monomer, (d) control.

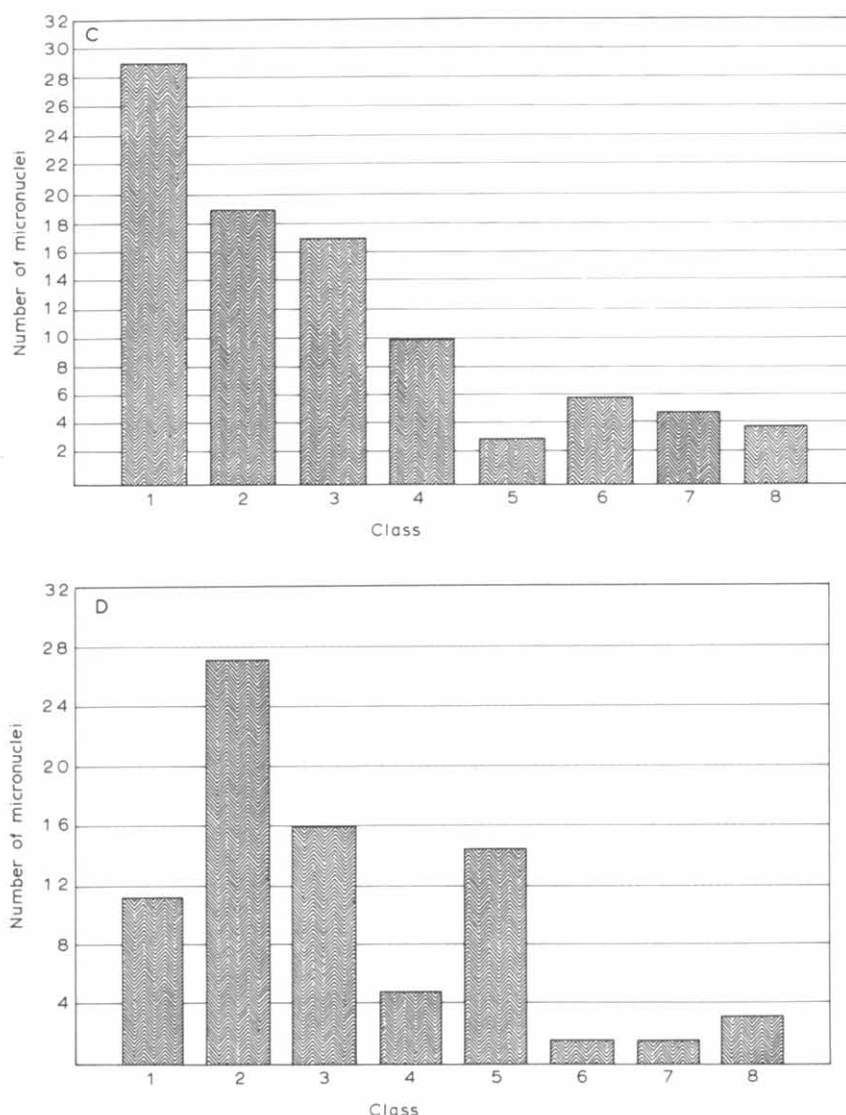


Fig. 1 (continued).

posed to X-rays had been exposed to doses lower than 25 mSv in the last 2 years. The dose was measured using a Siemens dosimeter. At the working places of the subjects exposed to pulsating microwaves, the power density of the electromagnetic field varied between $10 \mu\text{W}/\text{cm}^2$ and $20 \text{ mW}/\text{cm}^2$, with frequencies ranging from 1250 to 1350 MHz. The power density of microwave radiation was measured at the subjects' working places with a Raham, model 4A, which measures

the frequency range from 0.2 MHz to 26 GHz, and the power range from 0 to $20 \text{ mW}/\text{cm}^2$. The mean concentration of vinyl chloride monomer in the working environment measured by gas chromatography was 50 ppm; however, the concentration periodically reached 2000 ppm for a short period of time (4 h/w). The population for each agent had been employed for 15 years on average. All subjects were non-smoking males. The control analysis is based on non-smoking males,

25–45 years old (same age distribution as the workers).

For the micronucleus assay, cytochalasin B at a final concentration of $3 \mu\text{g}/\text{ml}$ was added at 44 h of the culture of lymphocytes, according to the method of Fenech and Morley (1985). Slides were made by fixation in 3:1 methanol:acetic acid without hypotonic treatment; the cell suspension

was dropped onto cold slides and stained in Giemsa. The surface areas of the micronuclei were measured by Morphomat 10. The surface areas were classified by size into 8 equal classes. The size of each class was defined as $1/8$ of the difference between the largest and the smallest micronuclei detected. The data on micronucleus surface areas were evaluated for statistical differ-

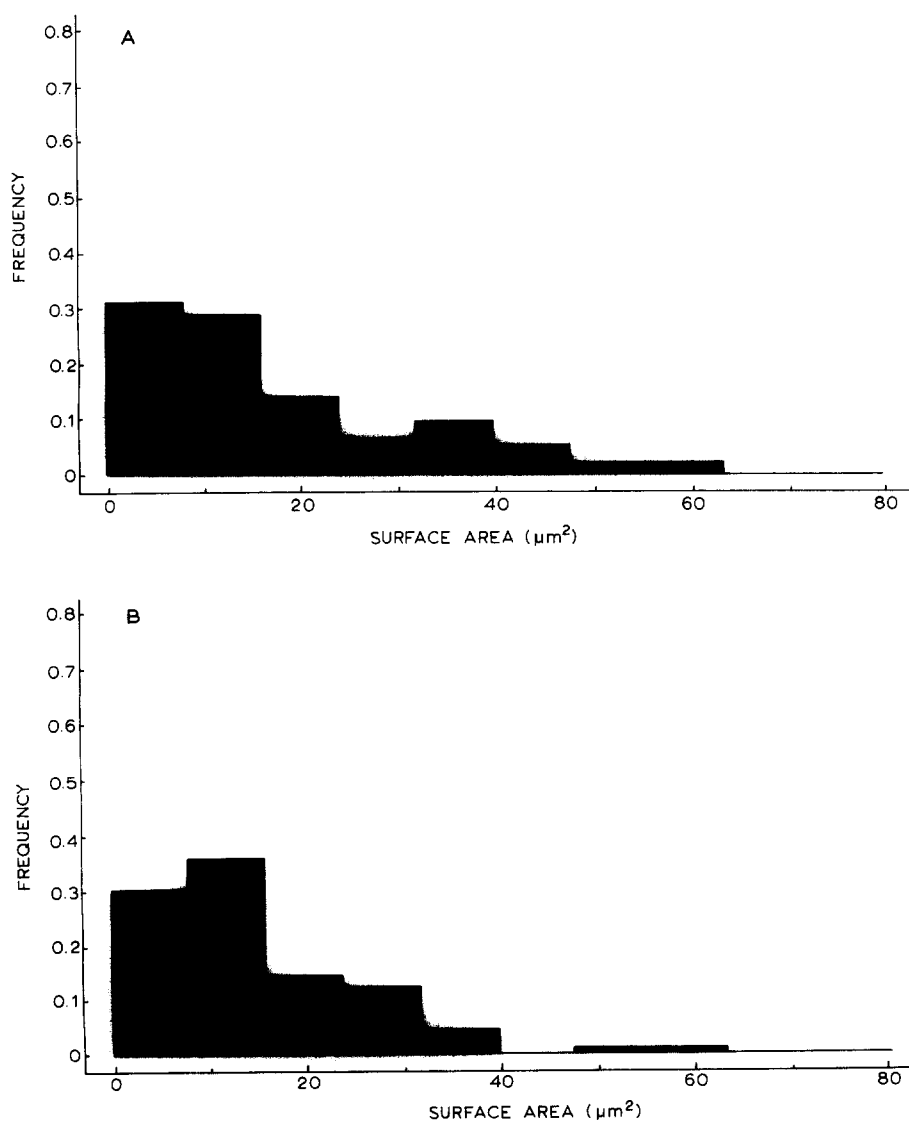


Fig. 2. Distribution of sizes of micronuclei in terms of surface areas. (a) X-Rays, (b) microwaves, (c) vinyl chloride monomer, (d) control. The difference between micronucleus surface areas for all three agents is statistically significant compared to the distribution of the control ($P < 0.001$). There is no statistically significant difference between distributions of micronucleus surface areas for microwave radiation and vinyl chloride monomer ($P = 0.181$).

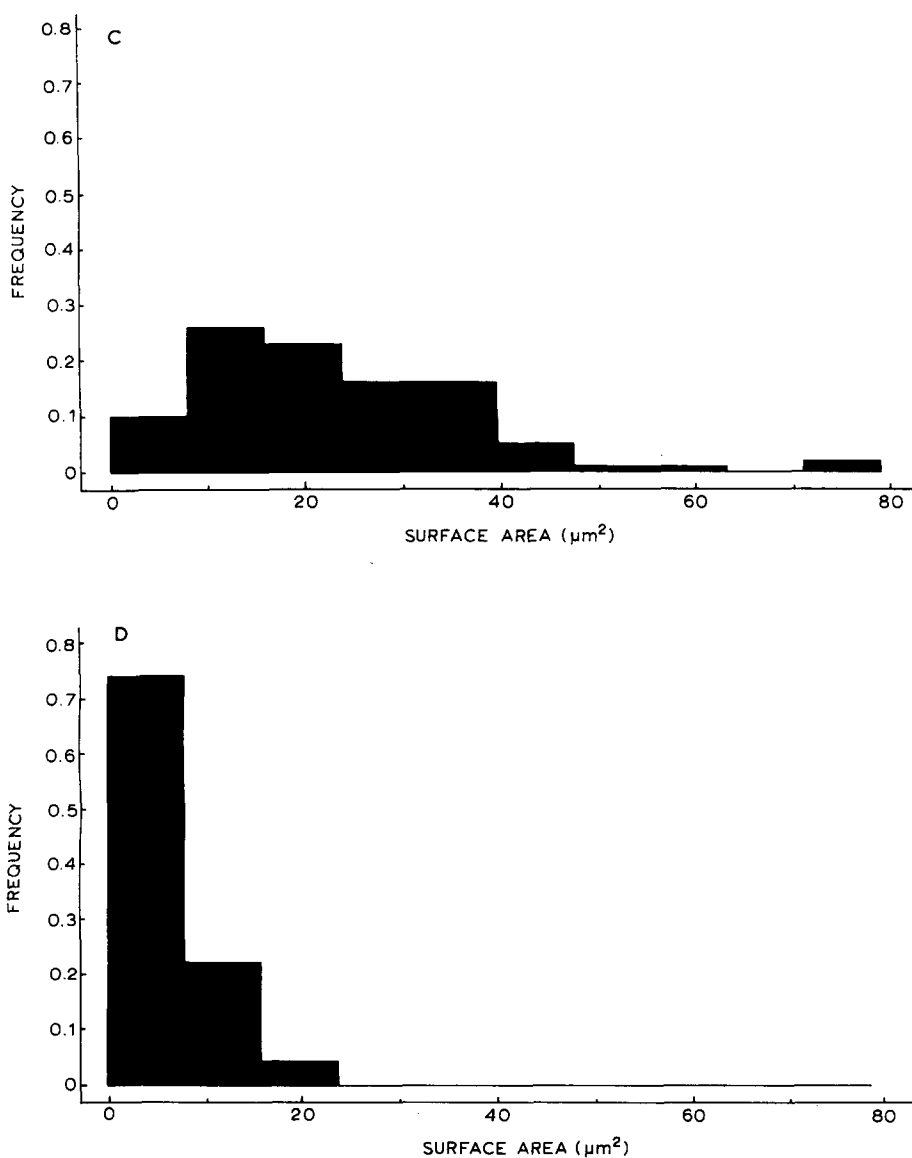


Fig. 2 (continued).

ence by the nonparametric Kolmogorov-Smirnov test (Sprenst, 1989).

Results and discussion

Vinyl chloride monomer, like the majority of chemical agents, produces predominantly chro-

matid breaks. Consequently most micronuclei are small in size and are distributed in the first three classes, indeed, 70% of the total values are located among such small micronuclei (Fig. 1c). However, this method allowed the detection of the existence of large micronuclei suggesting that vinyl chloride monomer can also be an aneugen. Such a distribution of large micronuclei is charac-

teristic of aneugenic chemicals that attack microtubulin (Vanparys et al., 1990).

Aneugenic activity correlates with the fact that vinyl chloride monomer metabolic products are alkylating agents (Osterman-Golkar et al., 1977) and that they react with nucleophilic sites in proteins as well as on DNA and thus may produce aneuploidy by the alkylation of spindle apparatus proteins (Dellarco et al., 1986) or guanosine triphosphate which is required for tubulin polymerization (Olmsted and Dorisy, 1975).

Chromosome breaks and DNA damage are considered to be the most frequent lesions produced by X-rays (Natarajan et al., 1986; Preston, 1982; Fenech and Morley, 1989). Their action as a producer of aneuploidy is thought to be rather weak (Eastmond and Tucker, 1989; Natarajan et al., 1986). The phenomenon that the micronuclei caused by the chemical mutagen vinyl chloride are smaller than those caused by X-rays could be explained by the fact that vinyl chloride produces chromatid breaks and X-rays produce chromosome breaks. This size difference is confirmed by Fig. 2, where the surface areas of micronuclei caused by X-rays (Fig. 2a) show bigger values than those caused by vinyl chloride (Fig. 2c).

There are several papers dealing with the final effect of microwaves on the genetic pool, but the molecular interactions which lead to this effect are still a matter of speculation. The distribution of the surface areas of micronuclei caused by microwaves may be related to the effects of ultrasound waves, which as a proved mutagenetic agent can, under certain conditions of frequencies and intensities, affect the biological material in a way similar to microwaves. Ultrasound has three categories of action: thermal effect, direct effect and cavitation (Baker and Darlympe, 1978; Galperin-Lemartre et al., 1975). The chemical effect arises from free radicals produced by the collapse of cavitation bubbles (Kondo et al., 1985; Galperin-Lemartre et al., 1975). Chromatid breaks caused by ultrasound can be suppressed by cysteamine. This suggests that ultrasound can induce the production of free radicals, while the mechanical effect arising from cavitation causes chromosome breaks (Fu et al., 1971; Doodly et al., 1984; Kondo et al., 1985). This architecture of the action of ultrasound overlaps with the picture given by the

micronucleus assay for genetic damage caused by microwaves. According to our research, microwaves have a dual effect on genetic material. As can be seen in Fig. 1b, the distribution of surface areas shows the characteristics both of a chemical agent (Fig. 1c) and of ionizing radiation (Fig. 1a). There is a large number of small and middle-sized micronuclei and a small number of large micronuclei. This is also the case with regard to ionizing radiation. The fact that there is no statistically significant difference between the distribution of micronucleus surface areas caused by microwaves (Fig. 2b) and vinyl chloride monomer (Fig. 2c) suggests the existence of a chemical effect of microwaves.

It has been observed that the number of micronuclei exceeds the number of chromosome aberrations (Fučić et al., 1990; Garaj-Vrhovac et al., 1991; Ford et al., 1988). This indicates that micronuclei do not originate from chromosome damage of any kind. The simple incorporation of acentric chromosome fragments into micronuclei may not actually account for the phenomenon. Micronuclei as a sign of action of some aneugens originate from chromosomes which fail to attach to the mitotic spindle (Fenech and Morley, 1985) as a result of damage to centromeres, centrioles or the protein components of the mitotic spindle.

In the present study, the effects of a chemical agent and radiation are for the first time compared by micronucleus assays on human populations exposed to these agents in their working environments. The size of the micronuclei is used to distinguish the ways of action of known carcinogenic pollutants. Our analyses show that although radiation has preferentially a clastogenic activity, its influences on the mitotic spindle are not completely absent. We provide evidence that vinyl chloride is not only a clastogen but also an aneugenic agent. In contrast to X-rays, microwaves exhibit a dual action, partly as radiation and partly acting as a chemical clastogenic agent.

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