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Chromosomal Aberrations in Onion (*Allium cepa*) Induced by Water Chlorination By-Products

Kabil Al-Sabti and Branko Kurelec

Center for Marine Research, The Rudjer Bošković Institute, 41001 Zagreb, Croatia, Yugoslavia

It has recently come to light that water chlorination generates mutagens and carcinogens. The water chlorination is not only responsible for the formation of a small number of volatile trihalomethanes, but also has the potential to produce high molecular weight nonvolatile mutagenic substances, organohalogens, derived from natural or man-made products (Jolley et al., 1980). The mutagenicity of nonvolatile mutagenic by-products of the water chlorination has been demonstrated in short-term biological testings, mainly in the Ames-Salmonella test (Loper, 1980), which supports the results of epidemiological studies showing an association of an increased cancer risk in humans with drinking waters treated with chlorine (Williamson, 1981; Kühn and Sontheimer, 1981; Cumming et al., 1980), and indicating environmental concern no. 1 - persistent organochlorines (Payne, 1981) - the by-products of the treatment of the cooling, drinking and waste waters with chlorine.

The predictive value of short-term tests, as all the protocols for the environmental assessment of genotoxic chemicals indicate, is considerably enchanced by the use of more than one test system. Scientifically most stringent approach in formulating a testing program for the assessment of genotoxins is to rely on tests of sufficient resolving power to detect a biologically singificant response, i.e., tests that directly measure gene mutations and chromosome alterations (ICPEMC, 1983). Chromosome aberrations (CA) become such a relevant bioassay. This biotest even introduces a new quality in the assessment studies: it measures the most important consequences of lesion of the primary target of mutagens, the DNA molecule - mistakes in its repair (misrepair) and the disturbance of the DNA synthesis in the S-phase of the cell cycle (misreplication) (Evans, 1977; Obe et al., 1982). The CA measurement in the Allium test is suitable for measuring the cytogenotoxic potential of chemicals present in waters: it is simple, cheap, sensitive, and it does not require a generally undefined step of concentrating chemicals present in polluted waters. Therefore, it was recommended by the Royal Swedish Academy of Science (1973) and by the GENE-TOX Program (Grant, 1982).

In the present investigation CA in Allium were chosen for the detection of mutagenic potential of a polluted river waters before and after the under-breakpoint chlorination.

MATERIAL AND METHODS

Small onion (Allium cepa) bulbs of the same uniform size, weighing about 4 g, were denuded by removing the loose outer scales and scraped so that the species of root primordia were exposed.

Water from the Sava river was collected daily for testing and chlorination from the banks near the Mladost bridge in Zagreb. Samples of water were analyzed within an hour after collection. In this work we used three samples (sample 1, 2 and 3). They were collected in September of 1983.

Colchicine and carmine were from Merck (FRG), benzidine and sodium hypochloride were from Kemika (Zagreb, Yugoslavia). "Aqualab" set for chlorine measurements was from Merck (FRG).

Freshly collected water from the Sava river was treated with the sodium hypochloride until 1 mg of residual chlorine was reached. The free and the bound chlorine were measured with the Merck Aqualab set. The chlorine demand of the three Sava water samples varied between 11.3 and 13.2 mg Cl/l. To eliminate the possibility of a direct effect of the free chlorine in the tests, the three chlorination treatments used in these experiments were below the chlorine demand level (1, 3 and 10 mg Cl/l) and before testing the chlorinated water was aerated for 30 min in order to decrease the amount of volatile trihalomethanes.

Tap water was dechlorinated by passing through the column of active carbon and as unpolluted it was used as control. Prepared onion bulbs were placed on the top of test tubes containing dechlorinated tap water. They were cultivated for two days (roots will grow 1-2 cm) and thereafter exposed in the dark at 20 °C to various polluted waters (the Sava water, the Sava water treated with 1, 3 and 10 mg Cl/l) for different periods of time. As negative controls we used dechlorinated tap water and an "exposure" of onions under the same conditions (darkness, time of "exposure"). Onions exposed to 1, 3 and 10 ppm concentrations of benzidine dissolved in the Sava water for 2 and 5 days served as positive controls. The above concentrations were prepared by dissolving benzidine in acetone and by a consecutive dilution in the Sava water so that the final concentration of acetone never exceeded 0.5%. Additional controls with 0.5% acetone in the Sava water were performed. For each test a group of three bulbs was used, so that the apices of their roots were prepared on one slide. The control contained three groups (slides), each representing 3 onions.

Chromosome preparation was done according to the protocol suggested by Grant (1982). Briefly: after termination of the treatment, bulbs were transferred to test tubes with 0.1% colchicine for 3 h

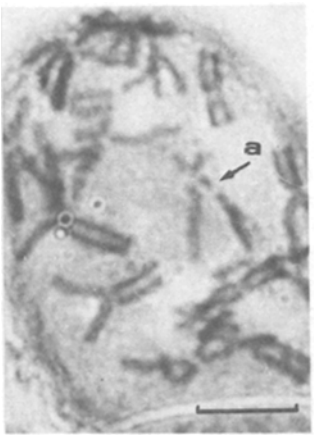

Fig. 1

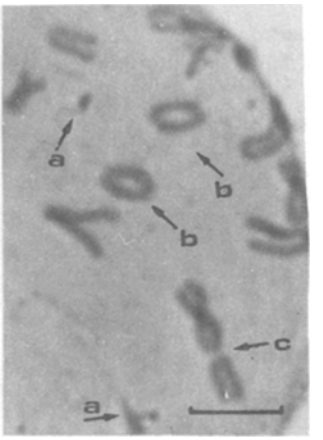

Fig. 2

Figures 1 and 2: Types of chromosomal aberrations recognized in this work. a.-breaks and fragments; b.-ring chromosomes; and c.-dicentric chromosomes. Scale: 10 um.

and thereafter to test tubes with distilled water for 1 h. After hypotonization (which is not in the protocol cited) the tips of the roots were cut and fixed for an hour at -20 C in the methanol glacial acetic acid (1:1). Staining was done with aceto-Carmine prepared as described by Romeis (1948). On the slides roots were cut into pieces, squashed with a cover slip and analyzed under the microscope (Opton, FRG) equipped with a built-in automatic camera.

We distinguish the following minimal types of aberrations: breaks and fragments, ring chromosomes and dicentric chromosomes. Regardless of whether there was a mono- or polyaberration in one mitosis, it was counted as one aberrated mitosis.

RESULTS AND DISCUSSION

The normal metaphases and different types of chromosomal deviations as classified in this work are shown in Figs. 1 and 2. As shown in Table 1, the control group has aberrations only in 10 out of 498 metaphases evaluated (2%) and these were found after only 24 h (4 of them) or 48 h (6 of them) of the root growth. Bulbs exposed to the Sava water (sample 1) show an incrased number of aberrations already after a 2 h exposure. After 48 h their frequency is above 10%. Chlorination of the Sava river water induces aberrations in a dose response manner with respect to both kinds of the "doses" - concentration of the chlorinated substances and the exposure time.

Even the lowest concentration of 1 mg Cl/l induces a higher number of aberrations than the unchlorinated Sava river water. It is interesting to note that response to the exposure is very fast, so that enhancement in induced chromosomal aberrations could be ob-

Table 1. Chromosomal aberrations induced by chlorinated river water

Treatment	exposure time in hours	no. of counted metaphases	% aberrations
Control (tap water)	2	140	-
	4	109	-
	24	117	3.42
	48	132	4.55
The Sava river water	2	157	3.82
	4	140	5
	24	156	8.97
	48	124	10.48
The Sava river water + 1 mg Cl/l	2	174	6.90
	4	151	8.61
	24	140	12.14
	48	119	15.13
The Sava river water + 3 mg Cl/l	2	164	9.15
	4	128	10.16
	24	111	16.22
	48	108	18.52
The Sava river water + 10 mg Cl/l	2	170	15.88
	4	148	17.57
	24	118	23.47
	48	106	25.47

served even after our shortest time of exposure. To further elaborate the time needed for the induction of CA, in a separate experiment we have exposed 2 days old roots to the chlorinated (10 mg Cl/l) Sava water (sample 2) and examined the appearance of CA in 15 min exposure intervals through the period of 90 min. Results are presented in Table 3. Two groups of bulbs exposed for 90 min were transferred to dechlorinated tap water for 2 and 4 h and examined for the frequency of CA which in these roots (Table 3) was found to be persistent.

Naturally, CA in the Allium roots exposed to the Sava river water are induced by unknown components of this composite sample. When such an "environmental" sample is chlorinated, then the identification of the active organochlorine component(s) becomes even more complicated. In order to somehow assess this obviously integrative biological response, a series of Allium were exposed to a known mutagen and carcinogen benzidine (Doll and Peto, 1983) for 2 and 5 days. For better comparisons, benzidine was dissolved in the Sava river water. The effects of the 2 day exposure (Table 2) could be directly compared to the variously chlorinated waters after the 48 h exposure from Table 1. The 5 day exposure to benzidine gives an insight into the magnitudes of CA which still allows the growth of the roots.

Table 2. Chromosomal aberrations induced by benzidine.

Treatment	exposure time in days	no. of counted metaphases	% aberrations
Control (tap water)	2	103	1.94
	5	90	3.33
The Sava river water	2	115	8.7
	5	546	12.33
The Sava river water + 1 mg benzidine/l	2	141	18.44
	5	138	24.64
The Sava river water + 3 mg benzidine/l	2	112	31.25
	5	132	40.15
The Sava river water + 10 mg benzidine/l	2	195	43.59
	5	185	65.95

Table 3. The dynamics of the induction of chromosomal aberrations and their irreversibility.

Treatment	exposure time in minutes	no. of counted metaphases	% aberrations
Control (tap water)	120	119	2.52
The Sava river water	120	121	3.31
The Sava river water + 10 mg Cl/l	15	132	9.09
	30	138	9.42
	45	102	9.42
	60	111	10.81
	90	117	14.53
	---------- transfer to tap water ----------		
	120	148	14.19
	240	151	16.56

Benzidine, which is mutagenic only after activation, induces CA in a dose-response manner even in the dark. The number of CA is higher than that induced by the corresponding concentrations of the chlorine used (concentrations of the organochlorines formed are not known) within 2 days of exposure. After 5 days of exposure to a concentration of 10 mg of benzidine/l, the CA reaches a very high number of 122 aberrated metaphases out of 185 (65.95%). Control onions, exposed to the initial concentration of 0.5% acetone did not differ in any way from the controls in the dechlorinated tap water.

It is interesting to note the frequency of appearance of different

types of aberrations induced by different treatments (Table 4). Both the organochlorines and the benzidine predominantly induce "breaks and fragments".

Table 4. Types of chromosomal aberration in percent after different treatments.

Treatment	breaks & fragments	ring	dicentric
Control (tap water)	50	20	30
The Sava river water	60	25	15
The Sava river water + 10 mg Cl/1	75	15	10
The Sava river water + 10 mg benzidine/1	90	5	5

The Sava river waters contain xenobiotics and, intermittently, mutagenic and/or carcinogenic substances (Kurelec et al., 1981). These waters greatly induce the MFO activity in the liver of the native or exposed naive fish (Kezić et al., 1983). When chlorinated, these waters become highly positive in both the direct and the Ames-microsomal test (Kurelec et al., unpublished data). In the under-chlorine demand treatment series, CA in Allium revealed a dose-response induction rate. Similarly, the induction rate of CA in roots exposed to one concentration shows a time-response dependency. The reaction time was very short: even the 15 min exposure to water chlorinated with 10 mg Cl/1 results in an increased induction of CA. An increase in CA induces already 1 mg Cl/1 after 2 h of exposure - the lowest concentration/time of exposure we have used. It was interesting to note that roots exposed for 90 min to 10 mg Cl/1 after the 2 or 4 h transfer to tap water did not recover - they have maintained the same or even induced a higher frequency of CA. At present we have no reasonable explanation for this fact.

The Sava river water per se, because of its low quality mentioned above, induces also an enchanced, time-dependent increase in CA.

The addition of benzidine, a strong mutagen that requires biotransformation (Garner et al., 1975; Lazear and Louie, 1977; Doll and Peto, 1983), strongly enhances the number of CA. This finding suggests the capability of Allium roots to metabolize benzidine. Similar ability of Allium to metabolize benzo(a)pyrene - another premutagen - was observed by Fiskesjö (1981). Only recently the mixed function oxydases (MFO) has been found in plant material (Plewa, 1978; Trenck and Sandermann, 1980; Plewa et al., 1983). This capability enchances the applicability and the predictive value of the test. The experiments with benzidine, a mutagen with a well-defined potency, offer a possibility of comparing the mutagenic potency of the Sava waters or its waters after chlorinat-

ion, and of expressing their mutagenic potency in something like "benzidine equivalents". Indeed, the use of benzidine in the Allium test, or other mutagens whose mechanisms of damaging the DNA are known and which consequently cause some type of CA, may serve as an indication that could, on the basis of the type of CA, define the damaging mechanism of environmental samples - as the rule a complex mixture of unknown mutagens, inhibitors, synergists and modifiers. Similarly to benzidine, our samples of chlorinated Sava river water prevalently enhance the breaks of chromosomes, which means that organohalogens cause mainly the double strand breaks of the DNA (Natarajan et al., 1982). Such CA are mostly responsible for the lethal effects (Scott and Zampetti-Bosseler, 1980; Zhestyanikov, 1982) resulting in various degrees of growth restriction of the Allium roots (Fiskesjö, 1979, 1981), while other CA can affect the vigour, fertility and reduced production of the exposed crop plant (Tomkins and Grant, 1976).

Since this plant material shows excellent correlations with mammalian systems (Grant, 1982), the results of the Allium CA measurements could be used as a relevant test for a general detection of genotoxins in the environment. As organochlorinated molecules are slowly biodegradable, in some percent even biorefractive, the results presented here point out at severe consequences of the widely used water chlorination and stress the environmental threat of organohalogens. The most important advantage of using the Allium CA lies in its extreme sensitivity allowing its direct application to polluted waters (or any other water in which some "material" could be dissolved). This eliminates the concentrating procedures needed in most analytical or biological analyses (Bedding et al., 1983). Thus, in addition to the information obtained by other biotestings, the induction of CA in Allium by organohalogens could bring some new insights into our understanding on the environmental consequences of water chlorination.

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REFERENCES

Bedding ND, McIntire AE, Lester JN (1983) Organic contaminants in the aquatic environment III Public health aspects, quality standards and legislation. Sci Tot Environ 27:163-200

Cumming RB, Becking KP, Cantor KP, Cotruvo JA, Kraybill HF (1980) Aqueous chlorination - Health effects, risk assessment and regulations. In: Jolley RL, Brungs WA, Cumming RB (eds) Water chlorination - Environmental impact and health effects. Ann Arbor Press, Ann Arbor, Vol 3, p 1141

Doll R, Peto R (1983) The causes of cancer. Oxford University Press, Oxford

Evans HJ (1977) Molecular mechanisms in the induction of chromosomal aberrations. In: Scott D, Bridges BA, Sobels FH (eds) Progress in genetic toxicology. Elsevier/North Holland, Amsterdam,

. p 57
Fiskesjö G (1979) Mercury and selenium in a modified Allium test. Hereditas 91:169-178
Fiskesjö G (1981) Benzo(a)pyrene and N-methyl-N-nitro-N-nitroso-quanidine in the Allium test. Hereditas 95:155-162
Garner RC, Walpole AL, Rose FL (1975) Testing of some benzidine analogues for microsomal activation to bacterial mutagens. Cancer Lett 1:39-42
ICPEMC (1983) Screening strategy for chemicals that are potential germ-cell mutagens in mammals. Mut Res 114:117-177
Jolley RL, Brungs WA, Cumming RB (1980) Water chlorination - Environmental impact and health effects. Ann Arbor Press, Ann Arbor, Vol 3
Kezić N, Britvić S, Protić M, Simmons JE, Rijavec M, Zahn RK, Kurelec B (1983) Activity of benzo(a)pyrene monooxygenase in fish from the Sava river, Yugoslavia: Correlation with pollution. Sci Tot Environ 27:59-69
Kurelec B, Matijašević Z, Rijavec M, Alačević M, Britvić S, Müller WEG, Zahn RK (1979) Induction of benzo(a)pyrene monooxygenase in fish and the Salmonella test as a tool for detecting mutagenic/carcinogenic xenobiotics in the aquatic environment. Bull Environ Contam Toxicol 21:799-807
Kühn W, Sontheimer H (1981) Treatment: improvement or deterioration of water quality? Sci Tot Environ 17:219-261.
Lazear EJ, Louie SC (1977) Mutagenicity of some congeners of benzidine in the Salmonella typhimurium assay system. Cancer Lett 4:21-25
Loper JC (1980) Mutagenic effects of organic compounds in drinking water, Mut Res 26:241-268
Obe G, Natarajan AT, Palitti F (1982) Role of DNA double-strand braks in the formation of radiation-induced chromosomal aberrations. In: Natarajan AT, Obe G, Altmann H (eds) Progress in mutation research, Vol 4. Elsevier Biomedical Press, Amsterdam, p 1
Payne JF, Rahimtula A (1981) Water chlorination as a source of aquatic environmental mutagens. In: Khan MAQ, Stanton RH (eds) Toxicology of halogenated hydrocarbons: Health and ecological effects. Pergamon Press, New York, p 209
Plewa MJ (1978) Activation of chemicals into mutagenc by green plants: A preliminary discussion. Environ Health Perspect 27: 45-50
Plewa MJ, Weaver DL, Blair LC, Gentile JM (1983) Activation of 2--aminofluorene by cultured plant cells. Science 219:1427-1429
Romeis B (1948) Mikroskopische Technik. R. Oldenbourg, München, p 153
Royal Swedish Academy of Sciences (1973) Evaluation of genetic risks of environmental chemicals. Ambio 3
Scott D, Zampetti-Bosseler F (1980) The relationship between cell killing, chromosome aberrations, spindle defects and mitotic delay in mouse lymphoma cells of differential sensitivity to X-rays Int J Radiat Biol 37:33-47
Tomkins DJ, Grant WF (1976) Monitoring natural vegetation for herbicide-induced chromosomal aberrations. Mut Res 36:73-84
Trenck T, Sanderman H (1980) Oxygenation of benzo(a)pyrene by

plant microsomal fractions. FEBS Lett 119:227-231
Williamson SJ (1981) Epidemiological studies on cancer and organic compounds in US drinking waters. Sci Tot Environ 18:187-203
Zhestyanikov VD (1982) DNA repair and cell repair. In: Natarajan AT, Obe C, Altman H (eds) Progress in mutation research, Vol 4. Elsevier Biomedical Press, Amsterdam, p 325

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