Investigation of toxic, mutagenic and cancerogenic effect of water disinfection by-products using *in vitro* and *in vivo* models

PhD theses

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CONTENT

LIST OF ABBREVIATION

ASV: Air saturation volume

EBA:4-etilbenzaldehide

EPA: Environmental Protection Agency

CDBP: Concentrate of Disinfection Byproducts

CSRW: Concentrate of Sediment of Raw Water

DBP: Disinfection byproduct

DFA:2,4-difluoroaniline

DOM: Dissolved Organic Matter

GC-MS: Gas chromatography–mass spectrometry

HAA: Haloacetic acid

HE: Hematoxylin-Eozin

IARC: International Agency for Research on Cancer

NOM: Natural Organic Matter

PAS: Periodic Acid-Schiff

PBS: Phosphate buffered saline

THM: Trihalometane

WHO: World Health Organization

INTRODUCTION

Consuming water is essential for human life.Due to environmental water pollutants – especially those produced by human actions – most of the natural sources of drinking water are polluted first of all by pathogenic bacteria causing severe epidenics. Water disinfection is widely used to avoid the harm caused by not-disinfected rough water.

Theprocess of treatingsurfacewatertorenderitsafefor human consumptionconsists of selectingthehighestqualityrawwatersource(s) available, adding a coagulant, flocculating, settling, filtering,and finally,disinfectingthewaterbeforestorageorpassagei intothedistributionsystem. Whileelements of thisframeworkhavechanged over timetoreflect technologicaladvancements, changesinregulations, and changingwatershedsorotherenvironmentalfactors, the overall treatmentobjectivesarethesame.

Sincetheirdiscovery, toxicologists and epidemiologists have beenconcernedaboutwater disinfection by-products (DBPs.) The occurrence of „highpriority"DBPsindrinkingwaterrraisesstill more questionsaboutthepotentialhealthimpacts of thesecompounds.

Although water disinfection by-products are harmful to human health, consumption of infected water is of higher risk. A good compromise should be reached regarding this problem. The aim of our studies was to investigate whether DBPs in drinking water samples in Hungary exert toxic, mutagenic or cancerogenic effects. Did we reach a really good compromise? This study is trying to answer the question.

AIMS OF THE STUDY

Considering data of the literature the following items were studied using water samples of water works in Budapest.

- the presence of DBPs in the drinking water
- the putative mutagenic effect of DBPs applying Ames test
- the putative apoptosis enhancing effect of DBPs on cultured human lymphocytes.
- the chemical components of the DBPs
- the putative apoptosis enhancing effect of of two among the more than 200 compounds found in the DBPs, selected on the basis of their chemical structure.
- the putative in vivo toxic or cancerogenic effect of the two selected compounds on Zebra Danio aquarium fish.

MATERIALS AND METHODS

600-800 litres of untreated (rough) water were taken from the ranneywells of the Csepel Subunit of the Budapest Waterworks. The same amount of disinfected water was obtained from the same subunit and was sampled at the end of water treatment process. Disinfection is being performed in this Subunit by means of chlorination and osonation. Chlorine and osone content of the water samples were determined by photometry and Iodomerty, respectively. LAB TOC 2100 device was used for controlling the organic carbonate content of the samples.

Identification ofunknown organic micro-pollutants of watersamples

The selected water samples were dropped permanently onto the special resin columns. The macroreticular resins Serdolit PAD III and Amberlite XAD-2 bindvariousways the organic micro-pollutants of the raw water and disinfected water. Theresin is white, pearl-shaped, hydrophobic, non-polar, non-ionic and, therefore,thenon-polar, non-ionic and neutralorganic compoundsare adsorbed,thepolarionicorganic compoundsare hardly bound.The organic compounds become enriched on the resin predominantly byadsorption, notby chemisorption, so the adsorbedcompounds can be deabsorbed with good efficiency.

Identification of unknown organic pollutants in water samples by GC-MS

Gas chromatographicseparationcombined withmass spectrometry (GC-MS) is suitable for characterization,identification ofthe quality of unknownorganicpollutants surface, groundwater, drinking waterandwastewater.

GC-MS test conditions

HP5890gas chromatograph was usedwith mass spectrometer ormass selectivedetectorwith the following settings: Injector 250 "C, splitless time: 1 min. Carrier gas: helium T5.5, head pressure 70 kPa; Colonna: chemically boundphase 100 metil- silicone[HP-ultra 1] length 25 m, internaldiameter 0.2 mm, filmthickness 0.50 µm; Detector: mass spectrometer (MS) SCAN operatingmode. Themass spectrometer will be set by so-calledauto-tuningtothetheSCANmethod before themeasure, than 1 µlsample and 1 µl blank is injected into thegas chromatograph, and record themass spectrum ofthe sample and theblank.

Evaluation of test results

Qualitativeidentification oforganicmaterials in thewater samplesandtheblindwas conductedin138Wileymass spectrallibraryby comparing thespectrum. Ifthe blank isunusuallydirty,needto checkon the preparation processstep by stepfurtherby makingblankto find outandeliminatethe cause of thecontaminationblind. Thedetection limit isabout1-10mg /L, depending on the quality of the compound andthedisturbingeffects.TheGC/MSmethodis notonlysensitive, butalsoenables cleridentification ofthematerial.

Genotoxicity tests

Ames test

The Ames Salmonella/microsomemutagenicityassay is a short-termbacterialreversemutationassayspecificallydesignedtodetect a wide range of chemicalsubstancesthatcanproducegeneticdamagethatleads togenemutations. The test employsseveralhistidinedependent Salmonellastrains eachcarryingdifferentmutationsinvariousgenesinthehistidineoperon.Thesemutationsactas hot spotsformutagensthatcause DNA damageviadifferentmechanisms. Whenthe Salmonella testerstrainsaregrownon a minimalmediaagarplatecontaining a trace of histidine, onlythosebacteriathatreverttohistidineindependence (his(+)) areabletoformcolonies. The number of spontaneouslyinducedrevertantcolonies per plate is relativelyconstant. However, when a mutagen is addedtotheplate, thenumber of revertantcolonies per plate is increased, usuallyin a dose-relatedmanner. The Ames test is usedworld-wideas an initialscreentodeterminethemutagenicpotential of newchemicals and drugs.

Many chemicals are not mutagenic (or carcinogenic) in themselves, but become converted into mutagens (and carcinogens) as they are metabolized by the organism. This is the reason why the Ames test includes a mixture of liver enzymes.Differentially labeledtestSalmonellastrainscan be used(eg,TA98, TA100), whichdifferinthe type ofthe most sensitivedetection ofmutations. Theassessment of the test consistsof countingandstatisticalanalysis of the revertantcolonies.

The assay is based upon the reversion of mutations in the histidine (his) operon in the bacterium. The his operon encodes enzymes required for the biosynthesis of the amino acid histidine. Strains with mutations in the his operon are histidine auxotrophs -- they are unable to grow without added histidine. Revertants that restore the His^+ phenotype will grow on minimal medium plates without histidine. Thereverse mutationandthemutation willbe formedin two ways: spontaneousandinducedbychemicals. The test isintendedto measurethe inducedmutationaleffects.

Apoptosis study

Venous blood of three, between 25-32 years old healthy non smoker adult men was taken using preservative K-citrate and processed within 1 hour. Mononuclear cells were isolated by the methods described by Boyum.

Flow cytometry

Attermination of theexperimentcellswerecollected and fixed in 70% ethanolat –20 °C for 24 h. Flow cytometry (DNA contentmeasurement) of lymphocyteswasperfomedusing a FACScan flow cytometer (Becton-Dickinson, Mountain View, CA, USA)inconjugationwith Macintosh Quadra computer and Cellquestdataacquisitionpackage. Estimation of theproportion of apoptoticcells and thecellcycleanalysiswereperformedusingModifit software. Cellsuspensionsampleswerepreparedasdescribedby Schuler*et al.* (1994). Briefly, afterethanolfixationtheinternucleosomallyfragmented DNA wasremovedfromapoptoticcellsincitrate-phosphatebuffer (pH=7.8) supplementedwithRnase, afterwards DNA contentwasdeterminedby flow cytometry. The control and threesamplesweremeasuredforeachdose.

Comet assay

The comet assay is a single cell gel electrophoresis test currently used as a qualitative and quantitative genotoxicity test. This is a simple and sensitive method for studying DNA damage and repair. In this microgel electrophoresis technique, a small number of treated lymphocytes suspended in a thin agarose gel on a microscope slide is lysed, electrophoresed, and stained with a fluorescent DNA-binding dye. Cells with increased DNA damage display increased migration of chromosomal DNA from the nucleus toward the anode, which resembles the shape of a comet. It is important toset thedensity ofagarose in order to enable wandering the 2-300kbp DNA fragmentswhile larger DNA fragments pathognomic for necrosis can not move from their embeddedposition. Followingrecommendations0.5%LMPagarosewas used.Cell membrane lysingis necessary because in the process of apoptosis the membraneremainsintactcomparedto necrosis.Thelysingsolutioncontains triton (NaCl, EDTA, Tris, DMSO, Triton), theprocess is at 4 ° Cin the dark for1 hour. Electrophoresis takes 15 min,with 300mA, 25V followedbystainingwithethidium bromide, and thefluorescenceorconfocalmicroscopicevaluation.Thetypicalapoptoticcellis comparable toacometwith atailcontaining thestainedfluorescentDNA fragments.

Acute Toxicity Test using zebrafish

The LC_{50} were determined using the OECD guideline [13] that describes the Fish Acute Toxicity Test. The stock-solution was 1000 mg/l.Two commercially available compounds, 2,4-difluorianiline (DFA) and 4-ethylbenzaldehyde (EBA) were chosen by gaschromatographic (GC-MS) analysis to start a series of *in vivo* studies.A semi-static test was applied, by changing the solution every 48 hours.The fish were exposed to the test substances for a period of 96 hours. Mortalities were recorded after 96 hours and the concentrations which kill 50 percent of the fish (LC50) are determined. Records were kept of visible abnormalities (e.g. loss of equilibrium, swimming behavior, respiratory function, pigmentation, etc.). Measurement of pH, dissolved oxygen and temperature were carried out at least daily.

Exposure and histology of zebrafish

In duplicate, fish cohorts were independently treated with two concentrations of DFA (2,4-difluorianiline) and EBA (4-ethylbenzaldehyde). The working solution contained 5 mg/l and 10 mg/l of DFA and 2.5 mg/l and 5 mg/l of EBA. Applied concentrations were administered *in situ* at levels determined to be at sub-acute levels, below LC_{10}, based on the previously determined LC_{50} values. Control groups, free of exposure to either compound were also generated in duplicate. Twenty-five adult fish, not differentiated by sex, were used in each replicate. The total density of fish was 0.4-0.5 g/l in each treatment.

Zebrafish were fixed in 4% buffered formaldehyde at 4°C for 24–48 hours, washed with Phosphate buffered saline PBS, and tissues were dehydrated in a series of graded ethanol solutions and xylene before embedment in paraffin. The fish were cut in half sagittally just left of the midline and both halves of the fish were placed into the cassette for sectioning. Sections were 4–6 μm thick and were stained with hematoxylin and eosin (HE), Periodic Acid-Schiff reaction (PAS), and Congo-Red.

Quantitative analysis of the effect of DBP exposure on fatty change of the liver using digital microscopy

Here, the effect of DFA and EBA were studied on the liver, using digital microscopy based on automated image analysisto detect and quantify changes in the amount on fatty degradation within hepatocytes. For each DBP, two different concentrations were used (5 and 10 mg/l for DFA and 2.5 and 5 mg/l for EBA). We exposed two groups of fish for each condition for the indicated time. Control groups were kept in DFA and EBA free medium. Random fields from the liver tissue were recorded and analyzed at 450x magnification to detect and quantify the area occupied by the non-stained lipid droplets within hepatocytes.

We generated digital slides from HE stained liver tissue of the studied fish. These digital slides are ideal to extract microscopic information at any magnification with easy navigation, annotation and measurement. Digital signals permit image segmentation along color, intensity, and size for automated object quantification while digital slides offer superior imaging features and batch processing. In this study we used the Pannoramic Viewer system developed by 3DHISTECH.

RESULTS

***In vitro testI.*:Mutagenic acticity of the untreated water samples**

The untreated well-water, concentrated on Serdolit PAD-III resin column, did not exert mutagenic effect on TA-98 tester strain neither with, nor without activation. However, mutagenicity could be observed after activation in case of the TA-100 tester strain. Very strong linear regression correlation was manifested between the number of revertant bacteria and the dose of water concentrates. The effect of these water sample concentrates fills the criteria of mutagenic activity. In case of samples gathered on Amberlite XAD-2 resin column, with and without activation of TA-98 and TA-100 tester strains. The summarised evaluation of mutagenic activity exerted on TA-98 tester strain. According to the data shown in the above motioned tables, the untreated well water concentrate was mutagenic to the tester strain TA-98, with and without activation.

The same concentrate showed mutagenic effect on the TA-100 tester strain only after activation. Again, very strong linear regression correlation was manifested between the number of revertant bacteria and the dose of water concentrates, fulfilling the criteria of mutagenic activity.

***In vitro testII*.:Mutagenic activity of the treated water samples**

The revertant bacterium number in case of TA-98 and TA-100 tester strains, with and without activation after treatment with disinfected water sample concentrates, if the concentrates were obtained using Serdolit PAD-III. Resin columns. The disinfected water showed negative mutagenic activity on the TA-98 tester strain when concentrated on Serdolit PAD-III. Resin column, with and without activation. The same concentrate showed positive mutagenic activity on the tester strain TA 100, but only after activation following extraction. The results of statistical analysis of the data show very tight linear regression correlation.

Positive mutagenic effect was found in case of TA-98 tester strain, with and without activation, when the concentrates of treated water were obtained using Amberlite XAD-2 resin column. According to the statistical analysis of the data, on the base of regression and strong correlation, positive mutagenic activity could be estimated. Mutagenic activity was observed also in case of the tester strain TA-100, with and without activation, when the concentrates of treated water were obtained using Amberlite XAD-2 resin column. The statistical analysis revealed linear regression, strong correlation, meaning mutagenic activity.

***In vitro test III.*:Apoptosisinductionwiththedilutions of water concentrates**

DMSO, as a single treatment increased the number of apoptotic cells. The increase was significant at 50µl/ml and strongest at 100µl/ml dose of DMSO. Since the water sediments and DBPs were dissolved in DMSO, the increase in apoptotic index exerted by treatment with these substances could only be considered as specific in case of the highest (100µl/ml) doses. The apoptotic index after 100µl/ml Concentrate of Disinfection Byproducts (CDBP)administration was strongly elevated and significantly higher than the values obtained by 100µl/ml DMSO and also by 100µl/ml Concentrate of Sediment of Rough Water (CSRW) treatment. CSRW, in 100µl/ml dose caused

increase in apoptotic ratio significantly higher than 100µl/ml DMSO.These results mean that both rough water and disinfected water contain substances which, in a sufficiently high dose, cause significantly strong elevation of the apoptotic index in cultures of peripheral blood lymphocytes.

Analyticalchemicalstudies: Identification of micro-pollutants

Based on the results of chemical analysis out of more than 200 compounds, twowere selectedon the basis oftheir chemicalstructure pointing to their assumed mutagenic, cancerogenic and apoptosis-inducing effects. The two compounds are 2,4-difluorianiline (DFA) and 4-ethylbenzaldehyde (EBA).

chemical structure of 2,4-difluoroanilin DFA (left)and4-etilbenzaldehidEBA (right)

Result of the Comet assay

Administration of DBPs, DFA or EBA resulted qualitative positiveComet test.

Apoptosisinductionoftwoselectedcompounds: 2,4-difluorianiline (DFA) and 4-ethylbenzaldehyde (EBA)

In our experiments,thedoses usedrangedfrom 1-200 mM for2,4-difluorianiline, and from 0,7-140 mM for 4-ethylbenzaldehyde. The cells weretreated with2,4-difluorianiline by doses above 10mM showedcytotoxic signs.Apoptosis in the 1-10mMdose rangewas observed. The 4-ethylbenzaldehyde treated cells showed cytotoxic effectover 7 mM, and apoptosis was observed in 0,7-7 mM dose range.

***In vivo test:*Zebrafish toxicity**

Histopathology

Alterations were found in the liver and kidney of the treated animals beginning in week 3 after the onset of the experiment. Among each high-dose exposure scenario (10 and 5 mg/l for EBA and DFA, respectively), the severity of augmentation increased gradually and reached its peak by the end of the second month. For the low-dose exposure scenario, however, effects were less consistent and seemingly time-independent among replicates exposed to 2.5 mg/l DFA and 5 mg/l EBA.

Liver alterations due to EBA exposure: within the liver parenchyma cells, changes were observed in the relative content and distribution of fat. The fat droplets varied in size, but at the experiments duration, nearly filled the whole cytoplasm. Furthermore, relative to the control, the glycogen content of the parenchyma decreased. These lesions were observed in both males and females. No hepatocyte megalocytosis, foci of hepatocellular alterations, or adenofibrosis were found.

Kidney alterations due to EBA exposure: HE and PAS stained sections showed small, clear, PAS positive vacuoles within the cytoplasm of the epithelial cells of the distal tubuli. Pycnotic chromatic condensations were found in 5-10 percent of these cells. Epithelial cells of the proximal tubuli showed larger, PAS positive, supranuclear droplets. However, the nuclei were without any observed alteration.

Liver alterations due to DFA exposure:throughout the study, diffuse fatty change was observed and most notable was the appearance of small fat droplets. The glycogen content of the liver parenchyma cells increased comparedto the control. No differences were observed in liver alterations between males and females. Preneoplastic alterations were not observed.

Kidney alterations due to DFA exposure: observedhistological changes were similar to those encountered with EBA.

Congo red staining for amyloid detection was negative in all organs of the fish treated with both EBA and DFA. No preneoplastic lesions or tumors of any kind were observed among fish exposed to either EBA or DFA.

Quantitative analysis of the effect of DBP exposure on fatty change of the liver

Low dose of EBA and DFA exposure did not change the fatty degradation of the liver parenchyma significantly ($P>0.7$ and $P>0.4$, respectively). However, high dose exposure to these DBPs caused significant elevation of the fat content of the liver cells.Thus, higher exposure concentration significantly increased the degree of fatty change of the liver cells. This difference between liver-alterations at low- and high dose DBP exposure can be explained by the detoxifying capability of liver-enzymes: only exposure to the high concentration level saturated the their enzymatic activity, resulting in the degradation that was noted.

Behavioral observations of zebrafish due to EBA and DFA exposure

In EBA exposure groups all fish were lethargic and did not evade capture. All fish exposed to DFA behaviour change was evident in the dominant observed swimming pattern and the display of behaviour associated with anxiety. DFA exposed-fish behaviour may be characterized by frequent and rapid changes in the direction of travel and was not observed among the control cohort.

DISCUSSION

Mutagenicity of untreated and disinfected water by *in vitro* Ames test

The mutagenic potential of water samples of various sources has been widely studied. Our previous investigations have shown mutagenic activity of drinking water concentrates derived from ground water wells and surface water. The possible mutagenic or even carcinogenic effect of drinking water on humans may be postulated on the basis of these results. However, systematic studies on the water concentrates obtained as a result of various forms of disinfection is needed in order to assess local or regional risks arising from consumption of drinking water. The disinfection procedure used by the water work Csepel, Budapest which provided the samples for our study consists of oxidation by O_2 and O_3, adding permanganate, flocculation, rapid sand and active carbon filtration and chlorination. The disinfection by-products resulting from this process were tested in our study.

According to our results, the concentrates obtained using Serdolit PAD III resin column were not mutagenic without activation, but after activation the concentrate of disinfected water showed mutagenic effect in case of TA-100 tester strain. On the other hand, DBP concentrates gathered on Amberlite XAD-2 resin column showed mutagenic effect with or without activation of the cultures in case of TA-98 tester strain. Activation was not needed for exerting mutagenic effect for the DBP concentrate, but concentrate of untreated water was ineffective without activation in case of TA-100 tester strain.

The data obtained using Amberlite XAD-2 resin column indicate, that DBP-s are present in the drinking water after application of the water-cleaning procedure.

It is noteworthy, that in our studies 0.83-2.5 liters of the disinfected water sample contained enough organic compounds to induce mutagenicity, whereas the same effect was produced by the amount of such compounds obtained from 0.28-0.83 liters of rough water. Thus, disinfection decreased the capacity of mutagenic compounds to a certain level, but did not eliminate these compounds.A significantobservation is thatthe treated, disinfected water'smutagenicratewas approximately three times higher comparedtothe untreatedwater.

Due toadverse healtheffects,it is absolutely necessaryto reduce the amount of DBPs produced by the disinfection. Search for alternative methods of water disinfection and continuous monitoring of the disinfected water regarding mutagenicity is recommended in order to improve the quality of drinking water.

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***In vitro*apoptosis-inducing effect of DBPs**

The presence of DBPs in drinking water has been known for a long time. The mutagenic and possible carcinogenic potential of these byproducts has also been demonstrated and discussed. However, no data in the literature are available on the apoptosis-inducing effect of DBPs. Our studies showed that water concentrates after disinfection and interestingly, also before disinfection exerted a dose-dependent, significant apoptosis-inducing effect when applied to cultures of human peripheral blood lymphocytes. In our previous studies a similarly dose-dependent mutagenic effect of similar water concentrates has been found.

It is well known that cells with damaged DNA may be eliminated from the cell population by mechanisms resulting in apoptosis. The question arises whether the

mutagenic and apoptosis-inducing effects are due to the same components of the concentrates.

The possible carcinogenic activity of the water concentrates with mutagenic and apoptosis-inducing effect should also be investigated. The results of such investigations may indicate whether the elevated apoptotic activity was sufficient to prevent the cumulation of cells with irreversible DNA damage.

On the other hand, increased apoptotic activity may cause tissue damages not related to carcinogenesis. Several alterations in the cardiovascular and nervous system are related to apoptosis of endothelial, myocardial cells or neurons. The possible contributions of the DBPs in the development of these alterations should also be taken into consideration. It is noteworthy, that concentrates of rough, non-disinfected water showed also significant apoptosis-inducing effect. It is unknown whether this effect is caused by products of bacteria or water-polluting compounds of various origin. Since only disinfected water is used for human consumption the further investigations should be directed to DBPs.

In vivo effects of compounds in water disinfection byproducts using the zebrafish model

In our *in vitro* studies concentration-dependent mutagenic effects of several DBPs were identified by Ames-test, and a similarly concentration-dependent, significant apoptosis-inducing effect of these DBPs appeared when incubated with cultures of human peripheral blood lymphocytes.

In the study at hand, an interesting *in vivo* vertebrate model was chosen to investigate possible toxic, mutagenic, and carcinogenic effects of two selected DBPs. The zebrafish has proved to be a good model system in which to study toxicology, carcinogenesis, and infectious disease and immune function. Moreover, zebrafish are easy to grow and care for and can be maintained inexpensively in large quantities. For histopathological analysis, the fish's small size allows examination of all organs with relatively few histologic sections placed on relatively few microscope slides. Moreover, the fish offer exceptional transparency which is an advantage for gross and stereomicroscopic examination.

When treated for three months with two doses chosen based upon acute toxicity, DFA and EBA did not induce lesions typical of carcinogen exposure in the liver of zebra fish. Extended exposure (one year to more years) to these compounds, however, could lead to evidence of carcinogenic activity. These experiments are in progress. Dystrophic lesions affecting the liver and the kidney were caused by both compounds, in both time- and dose-dependent manners. These lesions are not specific for either of the compounds, but drew attention to the possible toxicity.

The observed effects upon behavior and in response to external stimuli among zebra fish exposed to EBA and DFA also seems to be of considerable importance.

Since the behavioral effects exhibited by exposure to the two compounds are characteristically different and appear to impact the function of the fish nervous system in different manners, it is likely that specific modifying mechanisms are in the background. Such observations may be of interest also in context of human toxicology or even offer therapeutic considerations.

Since disinfection of drinking water is one of the most importance in the prevention of sudden, acute, and potentially fatal health endpoints, further studies into to the prevention of DBP formation or removal of compounds after formation are needed.

Following thorough analysis, the costs associated with the mitigation of DBP exposure can be coupled with the benefits associated with the prevention of undesirable health endpoints associated with deleterious components found in treated water as they are brought to attention in toxicology studies such as these. In the latter interest, further research on the in vivo effects of compounds in water disinfection byproducts using the zebrafish model is underway.

CONCLUSION

- The presence of water disinfection by-products (DBP) in drinking water in Hungary was first shown.
- These DBP-s exerted mutagenic effect by Ames test.
- The DBP- enhances apoptosis of cultured human peripheral lymphocytes (first published by us in the literature).
- Chemical analysis of the DBP-s revealed more than 200 compounds. Two of these were selected for further studies based on their chemical structure (2,4-difluorianiline (DFA) and 4-ethylbenzaldehyde (EBA)).
- Both DFA and EBA enhanced apoptosis of cultured human lymphocytes.
- Three-months administration of DFA as well as EBA to Zebra danio aquarium fish did not result in the formation of tumor sor preblastomatous lesions, but caused toxic liver and kidney lesions as well as abnormal reactions of the fis hon external irritation.
- These results endorse further studies on the biological damaging effects of DBP-s and extension of such studies on more chemical compounds found in the DBP-s

SUMMARY

The putativebiologicaldamagingeffect of waterdisinfectionby-products (DBP) wasinvestigatedregardingmutagenity, apoptogenity, toxicity and cancerogenitywiththefollowingresults.

- Drinkingwaterproducedbyone of the Budapest waterworkscontainedDBP-swithintherange of theinternationallypublishedquantities.
- DBP causedconcentrationdependent –positiveAmes test followingitsadministration to twobacteriumstrains, pointnigtomutagenicactivity.
- DBP administrationtocultured human peripherallymphocytescausedconcentrationdependentenhancement of apoptosis. Accordingtoourknowledgesimilarresultshavenotbeenpublishedintheliterature.
- More than 200 chemicallywell-definedcompoundswerefoundinthe DBP byourcollaborating partner. Out of thesetwowereselectedaspotentiallymutagenicorcancerogeniconthebase of theirchemicalstructure 2,4-difluorianiline (DFA) and 4-ethylbenzaldehyde (EBA) and were tested inourexperiments.
- Cultured human lymphocytesshoweddosedependentlyenhancedapoptoticactivityafter EBA or DFA administration.
- Threemonths DFA aswellas EBA administrationto Zebra danioaquariumfishresultedindegenerative alterations in theliver and kidney. Tumorsorpreblastomatouslesionswerenotfound.
- DFA and EBA causedabnormalitiesregardingthebehaviour of thefishcohorts.
- Long termadministration of DFA and EBA aswellas eother compounds found in the DBP to Zabra danios is plannad.
- The results of thepresent studiespointtothefactthatwaterdisinfectionby-productsprovedto be mutagenic, apoptosisenhancing and hepato/nephrotoxic. Theseeffectsmay be preventedoramelioratedbynewwaterdisinfectingmethodsorbyintroducingtechniquesaimingfurtherelimination of waterpollutants.

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