**FINAL REPORT SUMMARY**

**THE MECHANISTIC BASIS FOR PROVIDING A REALISTIC CANCER RISK ASSESSMENT FOR EXPOSURE TO INORGANIC ARSENIC WITHIN THE EUROPEAN COMMUNITY (ASRISK)**

Total project cost: 1 354 856 Euro    EU contribution: 1 354 856 Euro  
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**Objectives**

The overall objective for the investigations conducted under this project has been to improve existing risk assessments of inorganic arsenic (As). With this overall objective in mind, investigations were to be carried out with respect to exposed humans as well as in model systems.

The focus of the human studies was to characterize the pattern of As derived metabolites in human populations of different ethnicity, as well as to link the induction of chromosomal aberrations - the most sensitive toxicological index of exposure - to As exposure in drinking water. To be able to adequately assess the effects of As in humans, individuals should be identified who are exposed via the oral route at concentrations sufficiently high to elicit signs of As poisoning (>200 µg/day). The possibility of genetic differences in sensitivity, and given the fact that the current EU (WHO) risk assessment is wholly based on findings in a South East Asian population, would make it necessary to extend the scope for the project to areas outside EU.

The study of the mechanism of action is of cardinal importance for selecting the appropriate model for high-to-low dose cancer risk extrapolation, and mechanistic studies were to be conducted in cultivated mammalian cells to investigate the molecular mechanism(s) by which arsenic causes cancer initiation. The potential of As to enhance the risk for cancer due to the simultaneous exposure to other carcinogens by decreasing the capacity to repair bulky DNA-adducts was also to be considered.

**Results**

**Human studies** - The identification and characterization of exposed human cohorts suitable for sampling were directed towards an abandoned tin mining area at Ronphibun, Nakhon si Thammarat, Thailand, the Antofagasta area, northern Chile, as well as two cohorts in West Bengal, India. Relatively highly exposed subjects in Europe consisted of visitors to the Spa Bad Kissingen, Germany as well as workers exposed by inhalation in the Glogow copper smelters, Poland. From these cohorts, blood, and urine samples were collected.

As indicated by several indices of exposure, the highest intakes were detected in cohorts from West Bengal, where symptoms of chronic As poisoning were common, and similar high exposures were also documented for a few individuals from the abandoned tin mining area in Ronphibun, Nakhon si Thammarat in Thailand (urinary conc. up to 571 µg/L, median 85 µg/
Urinary concentrations of As among the Polish workers varied between 3 and 215 µg/l, where 14 individuals had urinary levels above 80 µg/L. For donors of Atacameña ethnicity selected from the Chilean settlements San Pedro de Atacama and Socaire, analysis of drinking water indicated very high levels of As (252-619 µg/L), but more recent intakes of As from these sources of water seem to have been low as judged by the urinary analysis, resulting only in a moderate to low excretion of As (median, 36 µg/l). Although the water from the German spa contained about 130 µg/L, urinary analysis indicated low actual daily intakes in the investigated 35 subjects.

Significant cytogenetic effects were observed in the cohorts in West Bengal, amounting to a 10-fold higher incidence of micronuclei in peripheral lymphocytes from subjects exposed to an average of 368 µg/L as compared to controls. The percentage aberrant cells was increased approximately four times in the second cohort exposed to well water with an average content of 212 µg/L. These latter values agree well with those previously reported by us for Atacameno Indians in the Salta province of Argentina, which indicated a LOAEL for MN induction at about 100 µg As/L in the drinking water. The Polish smelter workers with a mean urinary level of only 52 µg/L exhibited an approximately doubled incidence of MN. However, in view of the presence of confounding factors like exposures to other heavy metals and toxic materials, the interpretation of the Polish data in quantitative terms is somewhat uncertain. As could be expected from the low urinary levels, no significant elevation of micronuclei were seen for the Chilean cohorts, nor in the patients from the Bad Kissingen Spa.

Our experience from monitoring cohorts in different geographical regions demonstrate the difficulties associated with establishing the true extent of exposure, and the dangers of relying on drinking water analysis as the sole quantitative index of exposure in case of a waterborne pollutant.

**Experimental investigations** - Several studies have shown that bulky DNA adducts of unknown nature are formed endogenously in man. Using the postlabelling assay, we could demonstrate an increase upon exposure of mice to As(III). Studies in XPC repair deficient mice indicated that the repair status of the animals can modify the levels of these adducts, and that they accumulate with age. Extensive chemical investigations could not verify our original assumption that these adducts derive from 4-hydroxynonenal formed in vivo.

We have demonstrated that nucleotide excision repair (NER) is inhibited in human cells at physiologically relevant concentrations, and that incision appear to be the most sensitive step. However, several of the early steps of the formation of the incision complex seems unaffected, suggesting that the defect in the incision complex occurs at the later stages of NER. All repair proteins involved in NER up to the ERCC1/XPF recruitment appear to co-localise at local UV damage spots in the nucleus in As(III) treated cells, although DNA ligase I does not always co-localise with other repair proteins, such as RPA. Treatment with arsenic leads to a (partial) repair deficient condition in human cells after UV-irradiation.

In this context our finding that of a decrease of poly(ADP-ribosyl)ation in HeLa S3 cells after incubation with nanomolar concentrations of arsenite is of particular interest. Poly(ADP-ribosyl)ation is one of the immediate nuclear events following DNA strand break induction. PARP-1 is believed to mediate the main part of poly(ADP-ribosyl)ation of itself and various nuclear proteins involved in DNA synthesis, DNA repair and transcription.

Bypassing of lesions during replication may involve either homologous recombination or translesion synthesis (TLS). As(III) was confirmed to be able to induce homologous intra-chromosomal recombination in the hprt gene of Chinese hamster cells. Adducts induced by UV as well as by benzo(a)pyrene are affected by As to an equal extent in wild type Chinese hamster cells, indicating that NER is impacted in a general manner. In the NER deficient cell line UV5, a synergism was found between BPDE damage and arsenic, indicating that arsenic could be affecting a bypass process (e.g., trans-lesion synthesis, TLS). Support has been obtained for the hypothesis that interaction between stalled replication forks induced by arsenic and lesions originating from endogenous sources might recruit an error-prone repair complex (e.g., tolerance polymerases and/or recombination complexes) to the site of an endogenously induced adduct. This might result in a switch to a more error-prone pathway of repair of lesions generated from
one source that is potentiated by replication inhibitors from other sources. This is in agreement with the co-mutagenic properties of As as well as the finding by us and other researchers that As enhances DNA adduct levels from BPDE in the mouse. NER was shown to be involved in by-pass of bulky adducts induced by UV as well as those from BPDE.

In this project we have demonstrated that oxidative DNA damage is induced in vitro in HeLa S3 cells at fairly low non-cytotoxic concentrations of arsenite and by its pentavalent and trivalent methylated metabolites. Interestingly, the trivalent methylated metabolites monomethylarsonous [MMA(III)] and dimethylarsinous acid DMA(III)] reduced DNA repair at 5-10 times lower concentrations as compared with arsenite.

As (III) is slightly clastogenic in somatic cells of Drosophila, exhibiting a non-linear dose response. In this system the sensitivity of the SMART assay in response to UV was found to be increased when using the white/warts tumor suppressor gene variant. Deficiency in the XPF gene was found to cause a high degree of genomic instability as well as increased sensitivity to arsenite. Whereas arsenite was found to induce homologous intra-chromosomal recombination in the hprt gene of Chinese hamster cells, it did not increase the frequency of inter-chromosomal recombination in Drosophila.

**Benefits and Beneficiaries**

Occasionally, drinking water supplies in various areas of EU have been reported to contain As at levels that by far surmounts the current EU drinking water standard of 10 µg/L. Our investigations have also demonstrated that high occupational exposures to As by inhalation may occur in Polish copper smelters. In certain regions in Rumania, South Eastern Hungary, the Banat area of Vojvodina in Serbia as well as in Bosnia, the problems with markedly elevated levels of As in drinking water seem to be widespread, although systematic investigations are mostly lacking. Emissions and waste disposal associated with current or past mining, smelting, and refining of sulphide ores have resulted in widespread pollution in many areas.

Conflicting opinions exist with respect to risks associated with lower exposures that do not induce overt clinical signs of chronic arsenic poisoning, but there is little evidence to support the notion that the previous drinking water standard of 50 µg/L failed to offer sufficient protection. The current EU standard for As in drinking water relies on the corresponding WHO recommendation that was based on estimated theoretical cancer risks based on simplistic linear extrapolation. The supporting data base mainly derived from Taiwan, where the underlying exposure assessment was based on analysis of drinking water from selected wells. Our studies in Argentina, Germany, Chile and Thailand have demonstrated the dangers of relying on drinking water analysis as the sole quantitative index of exposure for a pollutant like As.

In accordance with existing EU Guidelines for cancer risk assessment, the ultimate aim of this project has been to define "virtually safe" levels based on mechanism of action. Results forthcoming from our project on arsenic have strengthened our previous surmise that arsenic induces neoplasia, not by interacting directly with DNA, but by affecting proteins involved in DNA replication and repair. This implies that the dose-response is indeed non-linear, implicating that a safe dose threshold in humans can be established. We have demonstrated that induction of micronuclei in human lymphocytes is apparently the most sensitive biological indicator of oral exposure to arsenic, and this parameter appears to be impacted at daily intakes above approximately 200 µg/day. The overprotective EU standard may be affordable to Western European countries with over-all low levels of arsenic in drinking water, and the Directive should be amended to state that no significant health risks can be foreseen when retaining the previous 50 ppb limit, while considering the new lower standard as a regulatory goal.
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