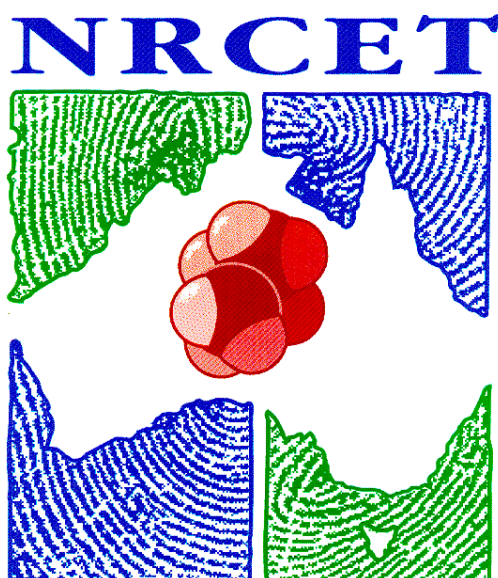


**EXAMINATION OF THE POTENTIAL EXPOSURE OF ROYAL
AUSTRALIAN NAVY (RAN) PERSONNEL TO POLYCHLORINATED
DIBENZODIOXINS AND POLYCHLORINATED DIBENZOFURANS VIA
DRINKING WATER**



A REPORT TO THE DEPARTMENT OF VETERAN AFFAIRS, AUSTRALIA

**THE NATIONAL RESEARCH CENTRE FOR ENVIRONMENTAL
TOXICOLOGY (ENTOX)**

QUEENSLAND HEALTH SCIENTIFIC SERVICES (QHSS)

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TO POLYCHLORINATED DIBENZODIOXINS AND POLYCHLORINATED
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A REPORT TO:

THE DEPARTMENT OF VETERAN AFFAIRS, AUSTRALIA

This report is dedicated to

Ralph Hayden Spooner

EX Ran Warrant Officer

Acknowledgements

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COMPOUND ABBREVIATIONS

I-TEq	2,3,7,8-Tetrachlorodibenzodioxin toxicity equivalents
PCDDs	Polychlorinated dibenzodioxins
TCDD	Tetrachlorodibenzodioxin
PeCDD	Pentachlorodibenzodioxin
HxCDD	Hexachlorodibenzodioxin
HpCDD	Heptachlorodibenzodioxin
OCDD	Octachlorodibenzodioxin
PCDFs	Polychlorinated dibenzofurans
TCDF	Tetrachlorodibenzodioxin
PeCDF	Pentachlorodibenzodioxin
HxCDF	Hexachlorodibenzodioxin
HpCDF	Heptachlorodibenzodioxin
OCDF	Octachlorodibenzodioxin
HCB	Hexachlorobenzene
DDE	Dichlorodiphenyl ethane
DDT	Dichlorodiphenyl trichloroethane
DDD	Dichlorodiphenyl dichloroethane
DMA	Dimethyl arsenic acid

EXECUTIVE SUMMARY

Studies of Australian Vietnam veterans have revealed greater than expected mortality, with the highest overall levels of mortality occurring among the Royal Australian Navy (RAN).

During the Vietnam War, large quantities of phenoxy herbicides (Agent Orange) contaminated with 2,3,7,8-tetrachlorodibenzodioxin (TCDD), arsenical herbicides (Agent Blue) and organochlorine pesticides were used. There has been concern that exposure to these chemicals may have long-term adverse health effects. TCDD for example is now known to have many toxic effects in humans, including carcinogenesis.

In RAN veterans, exposure to chemicals such as the TCDD is unlikely to be related to overhead spraying or other forms of direct contact.

The aim of this study was to investigate the potential for exposure of sailors to contaminants via drinking water. On Navy ships and Army small ships, potable water was produced from evaporative distillation of surrounding estuarine water. This water would have had variable salinity and amounts of suspended solids. It may have also contained contaminants in solution.

The study was carried out in two phases. First, the co-distillation of organic pollutants such as dioxins along with water in ship's distillation units was examined. Phase One results of this study demonstrated that:

- Co-distillation of organochlorine pesticides and dioxins was observable in all experiments conducted;**
- In pure or saline water, between 75% and 95% of 2,3,7,8-TCDD was co-distilled with the first 10% of water distilled. Thus, distillation results in an increase in the contaminant concentration in the distillate;**

- The tendency of several other organochlorines to co-distill was greater than for TCDD. For dioxins a tendency of decrease in co-distillation with increasing molecular mass was apparent. Hepta- and octachlorinated dioxins showed little tendency to enrich in the distilled water;
- A compounds' co-distillation decreased with increasing levels of suspended solids in the water. This can be attributed to the increase in sorption (fugacity) capacity in the source water. At a highest level of 1.44 g total suspended solids in the water about 38% of 2,3,7,8-TCDD co-distilled in the first 10% of water distilled. Nevertheless, even at these relatively high levels of suspended solids, TCDD was enriched by almost a factor of 4 in the distillate (assuming only 10% of the water is distilled);
- Co-distillation of dioxins and organochlorines from water collected from the Brisbane River (water was added to known amount of chemicals of interest) demonstrated that the process is reproducible using estuarine water. In these samples 48 – 60% of the TCDD co-distilled within the first 10% of distilled water.

Overall, Phase One of the study clearly demonstrated that if source water is contaminated, co-distillation is a process which can result in the contamination of ships water supplies with chemicals such as dioxins.

In Phase Two of the study the investigations included the potential co-distillation of the Agent Blue component dimethylarsenic acid, which is now known to be a potent carcinogen.

In addition, experiments were carried out in which the capacity for de-novo synthesis of dioxins from the main components of Agent Orange was evaluated. Evaporative distillation entails heating of the source water using copper elements. Combustion of the components of Agent Orange has great potential to produce dioxins. Moreover, copper (which formed part of the distillation unit) is a known catalyst for dioxin formation.

Finally exposure calculations were carried out for personnel on board ships. These calculations were based on some of the first analytical results from fish samples that were caught during the early 1970's in contaminated waters from Vietnam and analysed in the 1970's for TCDD.

Phase Two results of this study were:

- **Dimethylarsenic acid does not co-distill at significant levels during evaporation and thus the drinking water on board of RAN ships was unlikely to be contaminated with dimethylarsenic acid;**
- **No de-novo synthesis of TCDD or any other dioxins from the other components of Agent Orange was detected under the experimental conditions. However, the copper element on board ships was probably significantly hotter than in the simulation experiments selected in the laboratory, and thus these results should not be used as absolute evidence that such a formation did not occur in the distillation units of the RAN ships;**
- **TCDD exposure via drinking water may have been substantial, and it is likely that solely the consumption of drinking water resulted in exposure levels that exceeded the recommended Total Monthly Intake (TMI) values for TCDD of 70 pg / kg bw / month significantly. A TMI of 70 pg/kg bw / month is a level set by many European authorities; it is also the level proposed by the draft recommendation of the National Health and Medical Research Council in Australia.**

Overall the findings of this study demonstrate that evaporative distillation of water does not remove but rather enriches certain contaminants such as dioxins in drinking water. The study provides some evidence that use in the distillation process of water contaminated with TCDD would result in contamination of potable water. Subsequent ingestion by sailors on board ships (as well as

soldiers and airmen, who were passengers) is thus a vector for exposure to these chemicals.

While it is unlikely that accurate exposure of the personnel on board ships can be estimated, the study findings suggest that the personnel on board ships were exposed to biologically significant quantities of dioxins. This may explain some of the epidemiological findings in this study group.

INTRODUCTION

Studies undertaken by the Australian Government have indicated that Australian Vietnam veterans experience greater than expected mortality (Crane et al., 1997a) and that when mortality of two cohorts of conscripted veterans are compared, greater relative mortality (Crane et al., 1997b). Subsequent studies have revealed validated elevation in certain types of cancer in the veterans, and in their children, small increases in some birth defects and the rate of deaths, particularly suicide (AIHW, 1999).

The highest elevation in mortality was among veterans of the Royal Australian Navy, rather than the land and air forces (Crane et al., 1997a). Uncertainty remains as to whether this increase in mortality is related to the use of “Agent Orange” contaminated with polychlorinated dibenzodioxins and dibenzofurans (PCDD/Fs). In addition to “Agent Orange” and various other Agents that contained dioxin impurities, “Agent Blue” was the third most commonly used herbicide. It consisted of an aqueous solution of dimethylarsenic acid (DMA), or more commonly cacodylic acid. DMA was sprayed primarily in crop destruction missions (50%) or was used in the control of grasses around base perimeters. Recent research has demonstrated that DMA is a carcinogen itself (for details see Ng 2002, attached in Appendix II) and hence in Phase 2 this project was expanded to include preliminary evaluation of exposure to DMA.

A starting point of the study was that elevated levels of mortality were found in sailors. These sailors were never present in the areas where “Agent Orange” or any other Agents were directly employed for defoliation. Hence, prior to this study it was assumed that the significantly higher incidences of mortality observed in this cohort could not be related to the use of defoliants.

However, marine vessels such as the troop carrier and supply vessel HMAS Sydney served for substantial periods in estuarine waters in Vietnam and relied on collection of potentially contaminated estuarine water, which was then distilled for drinking. According to personnel on these ships a common procedure was to produce and store

drinking water from the relatively turbid estuaries, while the distilled water that was produced in open sea was primarily used for the boilers of the vessel's steam engines.

In the general population, exposure of humans to PCDD/Fs is attributed primarily to the consumption of contaminated food (e.g. Beck et al., 1989, Liem et al., 2000). This is due to the physico-chemical properties of these chemicals including their hydrophobicity and persistence and the resulting accumulation of these chemicals in lipophilic compartments of plants (Müller et al., 1997) and animals (McLachlan, 1996 1997).

These physico-chemical properties and in particular the exceptionally high sorption coefficients and very low solubility of PCDD/Fs in water are factors which reduce the risk of contamination of surface and ground water. Environmental fate models, as well as experimental evidence, suggest that consumption of contaminated water is a pathway which can safely be neglected in the calculation of exposure of humans to these compounds (Hattemer-Frey & Travis, 1989; Fürst, 1998, Liem et al., 2000).

However, in supply ships and other vessels which regularly visited the conflict areas in Vietnam, the water consumed by the crew has an unusual history. It was often collected from near-shore marine waters that received runoff from areas which had been sprayed with Agent Orange and Agent Blue. To make this water suitable for drinking and other purposes aboard ship the water was distilled aboard.

Evaporative distillation is a process that is suitable for obtaining water which is relatively free from salts and other high boiling components with a high water solubility. However, in contrast to their high sorption coefficient, both experimental data and models have indicated that the Henry's Law constant of chemicals such as dioxins is sufficient to allow desorption from natural water surfaces (i.e. Lyman et al., 1990). It seems feasible that co-distillation could occur during the distillation process on the marine vessels.

The aim of the study was to undertake laboratory experiments which provide information to assess whether PCDD/Fs and also DMA can co-distill in significant quantities in the distillation units of ships. Further, the study aimed to evaluate

potential exposure of PCDD/Fs to personnel aboard ships. The results are useful for an assessment of exposure pathways of PCDD/Fs to crew and troops aboard ships which regularly loaded sea water for distillation and subsequent consumption.

MATERIALS AND METHODS

Distillation Experiments with Dioxins and other Organochlorines

The project's goal was to identify whether significant quantities of potentially harmful chemicals may have co-distilled into drinking water in the ships which transported Australian Troops during the Vietnam conflict. The distillation plants used on the various ships at the time of the conflict all operated using the same principles. In general, sea water was fed into an evaporator where the water was boiled by a combination of heating and reduced pressure (vacuum) and the vapour was condensed in the condenser from where it was pumped into feed tanks (Figure 1). A detailed description of the operation and function of the distilling plants of ships is given in Naval Marine Engineering Practice Vol 1 (1959). The aim of this project was to reproduce the distillation plants principal processes in the laboratory and to assess the potential for co-distillation of chemicals in the distillation unit. Ultimately, this has provided information that allows us to evaluate the potential for contamination of drinking water from distillation of contaminated sea water.

Chemicals Tested

Agent Orange, the key defoliant used during the Vietnam conflict was contaminated with up to ~ 45 ppm of 2,3,7,8-TCDD and traces of 1,2,3,7,8-PeCDD (Young et al. 1978 quoted in IoM, 1999). It has been estimated that a total of 368 pounds of dioxin were sprayed in Vietnam over a six-year period (Gough, 1986 quoted in IoM, 1999). Although the defoliants did not usually contain relevant levels of any of the other 2,3,7,8-chlorine substituted PCDD/Fs, the study was extended to include a range of other 2,3,7,8-chlorine substituted PCDD/F congeners as well as the relatively nontoxic 1,2,3,4-TCDD and a range of organochlorine pesticides including DDT, HCB, lindane and dieldrin. This extension of the compound group allowed us to assess physico-chemical properties which govern the water volatility of lipophilic

Watervolatility of PCDD/Fs

organic chemicals and thus to predict water volatility for compounds which have not been studied here. In addition to the chemicals that were tested in Phase 1 of the project, we undertook further studies in Phase 2 of the project using DMA. A list of the chemicals used, including physico-chemical properties, is provided in Table 1.

Laboratory Distillation Plant

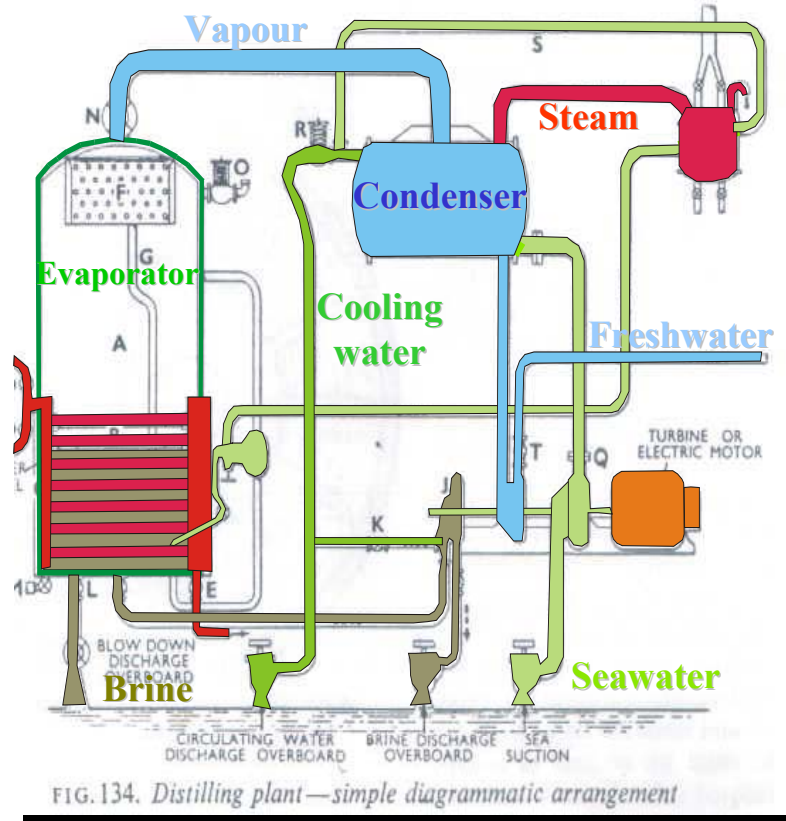
For the purpose of this study a commercially available rotary evaporator (Büchi, Switzerland) was used. Discussions with seamen and mechanics who served on RAN vessels during the conflict made certain that the principles by which solvents are evaporated in rotary evaporators were essentially the same as those used in the Naval Vessel Distillation Plants. Rotary evaporators such as the one used in this study essentially function as a batch evaporator. The water to be distilled is contained in a round bottomed flask with a seal which fits to the steam duct which leads the water vapour into the condensing chamber (Figure 2). The flask is lowered into a water bath which is maintained at the temperature of interest (in this study we used 58° C which is similar to that used in the distillation plants of the ships). In the condensing chamber, chilled water runs through a condenser coil and the water which condenses on the coils is collected in a solvent collection flask. The rotary evaporators are equipped with a pump, which is controlled through a vacuum control unit that allows accurate control of the vacuum during evaporation of the solvent. For the purpose of this study, the vacuum in the unit was set initially to 14 kPa (about 14 % of atmospheric pressure) and then slowly decreased until boiling of the water was observable. Water was then carefully evaporated since it was important to avoid non-vapour water containing the chemicals transferring through the condenser to the collection chambers ('bumping').

Distillation Experiments - Variations of Parameters

We studied the process through a series of experiments in which experimental parameters or compounds were altered. The key parameters which were altered were salinity of the water and quantity of suspended solids in the water.

For the experiments one litre round bottom flasks were cleaned with toluene and acetone and once they were dry, were spiked with a solution of the chemicals of interest. The round bottom flask was slowly swirled to coat the interior surface and to allow the solvent to evaporate. Once the solvent had evaporated, 1 L of reversed osmosis water (RO-water) was added and, depending on the variant of the experiment, known quantities of NaCl and/or sediment were added to the sample.

The round bottom flasks were then sealed, wrapped in aluminum foil and put on a shaker for at least one week so that the chemicals could equilibrate between the surface of the round bottom flask and the water.



Naval Marine Engineering Practice Volume I, 1959

Figure 1: Schematic diagram of the distillation unit on board RAN vessels

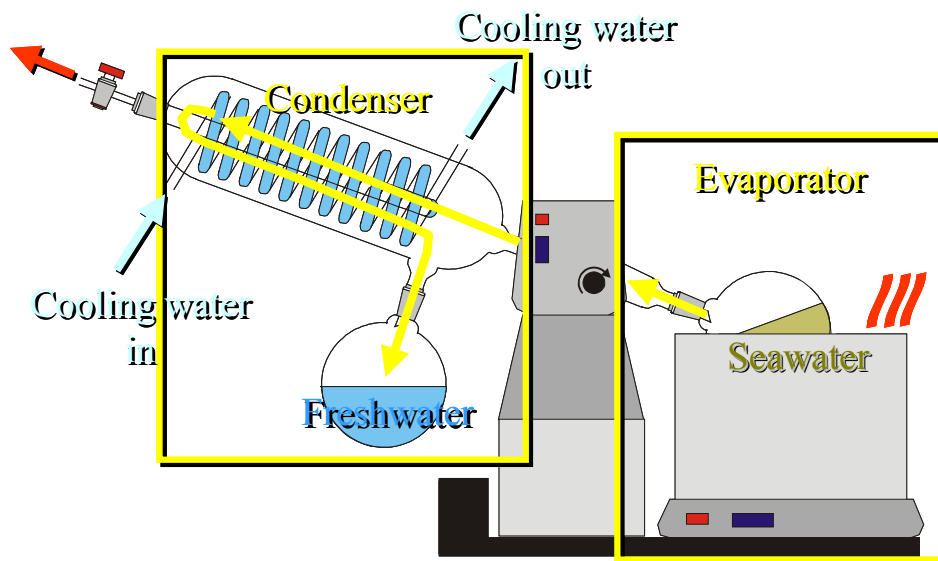


Figure 2: Schematic representation of the laboratory distillation unit

Table 1: List of chemicals used in the experiment, Molar mass, Henry's Law constant and water solubility data compiled from Mackay et al. 1992, Windholz, 1983, Paasivirta et al., 1999 and reviews by Huelster, 1994, Mueller, 1997, Cavanagh 2000 and IARC, 1987.

Compound	Molar mass (g)	Vapour Pressure* (Pa)	Aqueous solubility	H (Pa m ³ mol ⁻¹)	Spiked amount ng/L	RRT
HCB	284.8	0.0023	5 ug/L	131	100	0.36
Lindane	290.8	7-213 E-4	2.2 – 10 mg/L	0.005-1.5	100	0.50
Heptachlor	373.3	0.2 – 0.5	6 – 200 ug/L	18-233	100	0.54
Heptachlorepoxyde	-	3.5-450 E-4	20-200 ug/L	2-4.3	100	0.59
Aldrin	364.9	0.0008-0.75	10-200 ug/L	1.4-91	100	0.73
Dieldrin	380.9	0.2-9 E-4	20-2000 ug/L	0.02-5.8	100	0.82
DDT	354.5	0.2 – 20 E-4	1-460 ug/L	0.86-7.3	200	0.86
DDE	318.1	1.7 – 10 E-4	1-55 ug/L	0.8-124	200	0.98
DDD	320.1	1-9 E-4	2-160 ug/L	0.27-9	200	1.02
2,3,7,8-TCDD (D4)	322.0	1.2 – 6.2E-4	8-200 ng/L	3.347	40	0.99
1,2,3,4-TCDD	322.0	6.38E-6	640 ng/L	3.8	8.6	1.14
1,2,3,7,8-PeCDD	356.4	4.23E-6	120 ng/L ^a	0.266	40	1.18
1,2,3,4,7,8-HxCDD	391.0	1.45E-6	4.4 ng/L	1.084	40	1.36
1,2,3,4,6,7,8-HpCDD	425.2	1.77E-7	2.4 ng/L	1.273	40	1.61
OCDD	460.0	1E-10 -9E-7	0.074-0.4 ng/L	0.684	60	1.96
2,3,7,8-TCDF	306.0	1.2 – 2E-4	419 ng/L	1.461	40	1.00
1,2,3,7,8-PeCDF	340.4	1.72E-5	236 ng/L ^b	0.505	40	1.14
1,2,3,6,7,8-HxCDF	374.9	3.08E-6	17.7 ng/L	1.454	37	1.31
OCDF	443.8	5.0 E-10	1.4 ng/L	0.191	61	1.95
DMA	138.0	n.a. but low	2 kg/kg	n.a. but very low	1000	n.a.

RRT was calculated from retention times on a DB1 column; *vapour pressure data represent subcooled liquid vapour pressure's; ^a for 1,2,3,4,7-PeCDD; ^b for 2,3,4,7,8-PeCDF

For the volatilization study, the rotary evaporator was disassembled and all sections which could come into contact with the chemicals were thoroughly cleaned to avoid contamination of the samples. In order to determine the quantity of water which had been distilled, the mass of the collection flask was determined before the start of the experiment. The temperature was controlled through a water bath which was set to 58⁰ C as described in the manual for the ships distillation unit. In all experiments the goal was to slowly distill a fraction of the water and evaluate the amount of dioxins and organochlorines which co-distilled. Although in the initial proposal it was only proposed to analyze one distillate it was decided to distill two fractions, a first fraction of about 10 % of the water and a second fraction with about a further 30 % of the water. For the distillation process the round bottom flask was attached to the rotary evaporator and the vacuum in the system was increased until about 13 - 14 % of atmospheric pressure (14 kPa) was reached. The flask was rotated to increase the surface area of the water to be distilled and to avoid 'bumping'. Chilled water (10⁰C) was circulated through the condenser unit. The experiment was carefully observed over the first few minutes until the water temperature in the flask had increased to the assigned temperature to avoid 'bumping'. Once the system temperature had equilibrated the vacuum was carefully decreased to a pressure which resulted in a slow and steady distillation of the water. Markings on the collection flask allowed a rough assessment of the quantity which had been distilled and in the distilled fraction 1 (F1) about 10 % of the water and in the distilled fraction 2 (F2) a further 30 % of the water was collected (For details of the collected fraction see Appendix I Table 3.). Following the distillation of the first fraction F1, the inside of the condenser unit of the rotary evaporator was rinsed with RO water which was added to the F1 fraction. Once the second fraction, F2, was distilled the inside of the rotary flask was rinsed with about 10 mL of acetone followed by dichloromethane, both of which were added to F2.

Following the distillation the various fractions F1, F2 as well as the remaining non-distilled water (R) were transferred into separating funnels and subjected to liquid-liquid partitioning using dichloromethane and hexane. The nonpolar fractions were combined and concentrated to a small volume (< 500 µl). In the preliminary

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experiment and Expt.1 the samples were quantitatively transferred into 50 μl microvial inserts, concentrated under a gentle stream of nitrogen to almost dryness and filled with 15 μl of toluene.

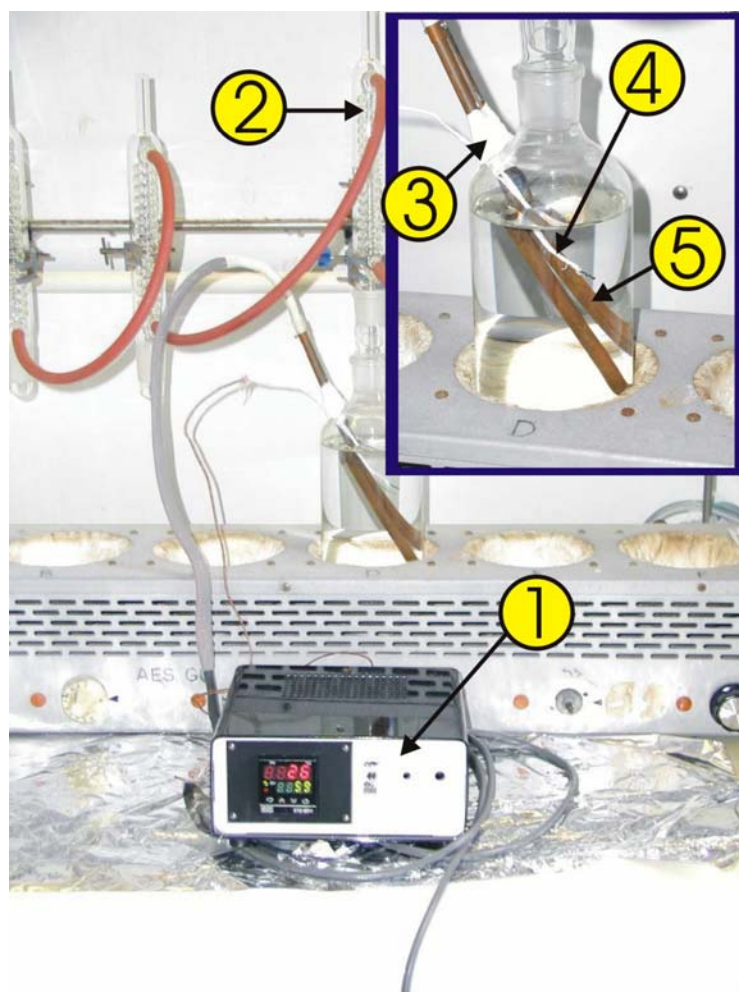
For Experiment 2 it was decided to include a clean-up step using H_2SO_4 and KOH impregnated silica gel in series in a Pasteur pipette. Samples were eluted using hexane, the hexane was evaporated and the samples were transferred into vials, concentrated and filled with toluene as described above.

Since it was of great relevance to this study to detect congeners in all three fractions (F1, F2 and R) to undertake the mass balance type approach we decided to use the most sensitive tool available for this study. The fact that known quantities of standards were added allowed the use of gas chromatography coupled to electron capture detection where the sample was injected onto columns of varying polarity. The results from the experiment indicated that mass spectrometric quantification was sufficiently sensitive for this study. Hence further analysis of PCDD/Fs and organochlorines were performed on a Gas Chromatograph (DB-5 fused silica column, 30 m, 0.25 mm i.d., 0.24 μm film thickness) interfaced to a quadropole mass spectrometer operating in selective ion monitoring mode. Organochlorines and PCDD/Fs were identified using retention times in the standard solution and evaluation of correct isotope ratios M^+ and M^{2+} . Quantification was undertaken by external calibration against some standard used to spike the samples. (Note that the study did not require absolute quantification of the concentrations since the aim was to evaluate the relative proportions of the chemicals of interest in various fractions of the distillate.)

Formation Experiments

In order to evaluate “de-novo” formation of dioxins in the distillation unit itself from precursors, a system was developed in which an electrical heating element was inserted into copper tubing to represent the heating element in the distillation units aboard ships which also consisted of copper tubing (Figure 3). The element was operated in connection to a thermocouple so it could operate at a water temperature of 55°C , which was similar to that in the ship’s distillation unit.

The copper coated element was then mounted at an angle into glass jars that were specifically designed to allow the element to be sealed inside while a condenser was mounted onto the top to make certain that the distilled water was reused in the flask. The formation experiments were conducted using the Agent Orange components 2,4,5-T and/or 2,4-D, which could act as a precursor for formation of TCDD and PeCDD. In the formation experiment, empty flasks were spiked with precursors, then RO water and additionally 30 g NaCl was added to obtain a salinity level similar to that of an outer estuary. The water was then equilibrated for 5 days or more. The copper coated heating element and thermocouple were then inserted into the solution and the opening sealed with Teflon tape. Finally, the condenser was inserted into the top of the flasks and the thermocouple was set at 55°C in the outer periphery of the flask. The formation experiments were carried out for 12 hours. In addition to the test samples, blank samples containing 2,4,5-T and/or 2,4-D were added to water but not heated, and a blank consisting only of water were also included.



1. Temperature Controller
2. Condenser
3. Teflon Seal
4. Thermometer
5. Copper header

Figure 3: Experimental set-up of the formation experiments. Agent Orange components were spiked into the glass vessel and heated up using the copper coated heating element.

RESULTS AND DISCUSSION

Blanks – Distillation Experiments

The series of blanks included in the study were: undistilled RO water, RO water with suspended solids, NaCl and water from the Brisbane River. These were analysed together with fractions F1, F2 and R. None of the compounds of interest were detected in any of the “blank” samples which were not spiked with the SPIKE standards used.

Co-distillation of Dioxins and Organochlorines from Pure Water

In Experiment 1 the emphasis was on evaluating the distillation method of RAN vessels and to determine whether co-distillation is observable. This was initially tested in the simplest experimental configuration where organochlorine pesticides and dioxins were added to a flask, equilibrated with RO water and then the sample was distilled. In the first experiment 14.1 % of the water was distilled and the distilled water was analysed for the compounds initially added. For this initial experiment the remaining water was analysed but either due to loss of the compounds, during the extraction, interferences and/or absence of the compounds no significant quantities could be detected in the second distillate and the remaining fraction. The results are thus comparable with a water sample which was spiked and equilibrated but not distilled and where the compounds were re-extracted from the initial water sample.

The results of the first distillation experiment are shown in Figure 4. These results clearly demonstrated that organochlorines and dioxins have the potential to co-distill relatively rapidly. Overall, the relative quantity of the chemicals of interest in the initially distilled fraction, compared with the concentration in the non-distilled water, ranged from 113 % to about 13 % (Appendix I, Table 1). Co-distillation was greatest for the organochlorine pesticides and lower chlorinated PCDD/Fs. These initial results suggested that almost the entire quantity of chemicals such as hexachlorobenzene or gamma hexachlorohexane and about 73- 85 % of the DDTs co-distilled with the first 14 % of water. For the polychlorinated dibenzodioxins and dibenzofurans there was a

trend to decrease co-distillation with increase in chlorine number. Interestingly in this first experiment 2,3,7,8-tetrachlorodibenzodioxin, the most toxic of all PCDD/Fs and the main contaminant in Agent Orange, was found at about 85 % of the quantity observed in the nondistilled samples and thus co-distilled the most. In contrast only about 10 – 20 % of the hepta- and octachlorinated PCDD/Fs were found in the distilled fractions in the first experiment.

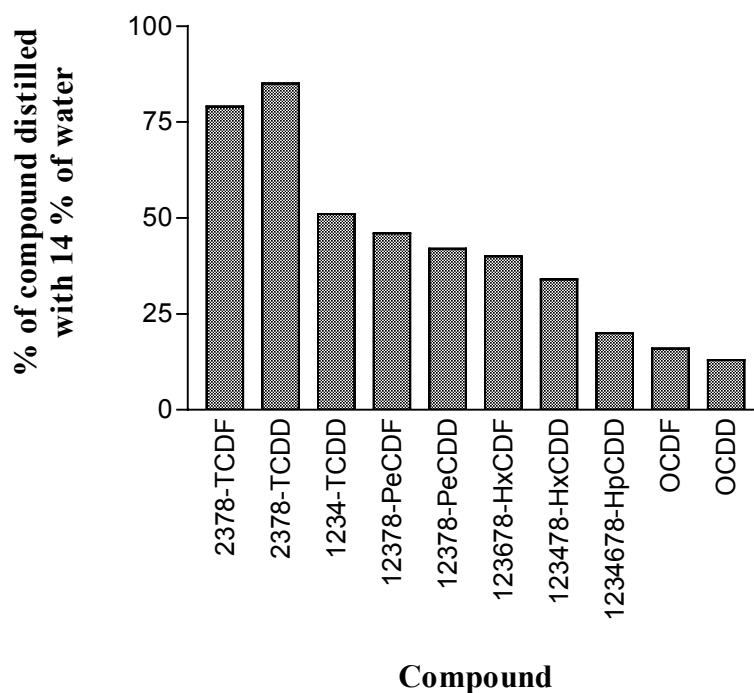


Figure 4: Co-distillation of organochlorines from pure RO water when 14 % (141 mL of 1000 mL) was distilled. The values represent the quantity in the samples distillate relative to the total quantity in a control of spiked but non-distilled water.

Effect of Salinity on Co-distillation of Organochlorines

The effect of salinity on co-distillation was assessed in Experiment 1 by evaluating the co-distillation of dioxins and organochlorines at varying NaCl concentrations. In addition to the control (with no NaCl) 15 and 30 mg/L NaCl treatments were distilled after the equilibration period and the different fractions analysed. As observed in DVA1-1, DVA1-2 and DVA1-3 organochlorines distilled very quickly and were largely associated with the initial fraction of the distilled water (Appendix I, Table 1). This was also the case for 2,3,7,8-TCDD of which ~ 95 and 87 % were found in F1 of DVA1-2 and DVA1-3 respectively. In order to compare the results, the relative fraction of the chemicals co-distilled in the first fraction were plotted against the relative retention times (RRT) of the chemicals on a DB5 capillary GC column where the reference value of 1 was attributed to 2,3,7,8-tetrachlordibenzofuran (See Table 1) (Note that the RRT of chemicals were determined in Experiment 1 and can be related to physico-chemical properties of the compounds of interest such as water solubility or vapour pressure, both of which are likely to influence the water volatility of hydrophobic organic chemicals significantly). Due to uncertainties in the accurate determination of the most relevant physico-chemical properties which govern water volatility (i.e. water solubility, vapour pressure or in combination as Henry's Law Constants) the authors have not plotted co-distillation against these properties although it is clear that these govern the water volatility. To avoid the process of selecting the "best" value from the literature the authors have decided to use the RRT which is an easily obtainable measure and is also related to the relevant physico-chemical properties of compounds. Overall the organochlorines have a RRT less than 1, and the RRT of PCDD/Fs increases with increasing molar mass).

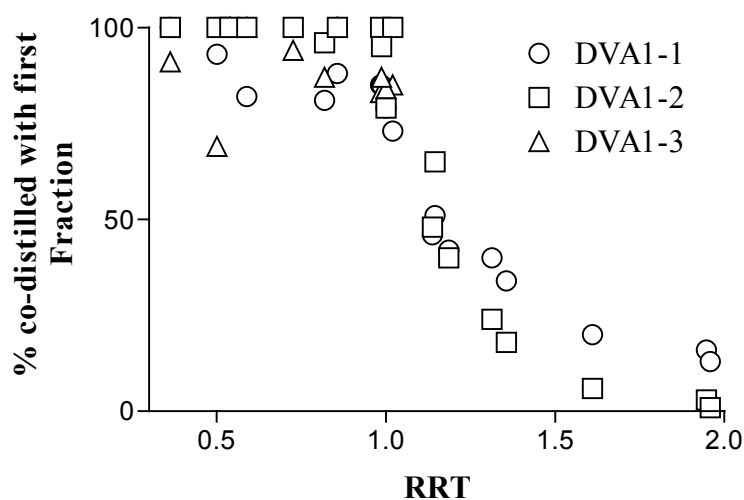


Figure 5: Plot of the relative retention times of organochlorines and PCDD/Fs versus the % co-distilled in F1 at various NaCl concentrations (DVA1-1: 0g NaCl L⁻¹; DVA1-2: 15g NaCl L⁻¹; DVA1-3: 30 g NaCl L⁻¹)

The data obtained in Experiment 1 were not suitable to quantify the effect of salinity on the co-distillation of the compounds of interest (Figure 5). The differences would be observed best with the compounds which did not rapidly co-distill but overall showed a good tendency for co-distillation. Unfortunately, interferences in the chromatogram from DVA1-3 (30g NaCl per litre) did not allow us to interpret the data from those compounds. Further uncertainties arose from differences in the amount of water which was distilled at the three NaCl levels. The interpretation was complicated by the fact that some chemicals such as the organochlorines of interest or lower chlorinated PCDD/Fs volatilise relatively quickly and are thus depleted in the remaining water phase (R). Therefore, the quantity which co-distills decreases with longer distillation times. In contrast, chemicals such as the heptachlorinated dibenzodioxins and octachlorinated dibenzodioxins and dibenzofurans concentrate in the R phase with ongoing distillation and a slight increase of co-distillation with increasing distillation can be expected. In order to evaluate this, a second fraction F2 was distilled in addition to the first fraction F1. The ratio of F1 to F2, normalised to the amount of water distilled, provides an indication of the extent to which co-distillation is decreasing/increasing with increasing distillation of the water. Chemicals for which co-distillation is more rapid than the distillation of water and

which are thus depleted should be relatively enriched in F1 compared to F2. A plot of the RRT versus the ratio of F1/F2 demonstrates that with increasing RRT the ratio decreases from > 40 for chemicals such as HCB and approaches 1 for the higher chlorinated PCDD/Fs (Figure 6). This suggests that most chemicals investigated were already depleted during the distillation of F2 and only the higher chlorinated PCDD/Fs have lower concentrations in the distilled water than in the source water if the sorption compartment in the water (i.e. organic matter and suspended solids) is very low.

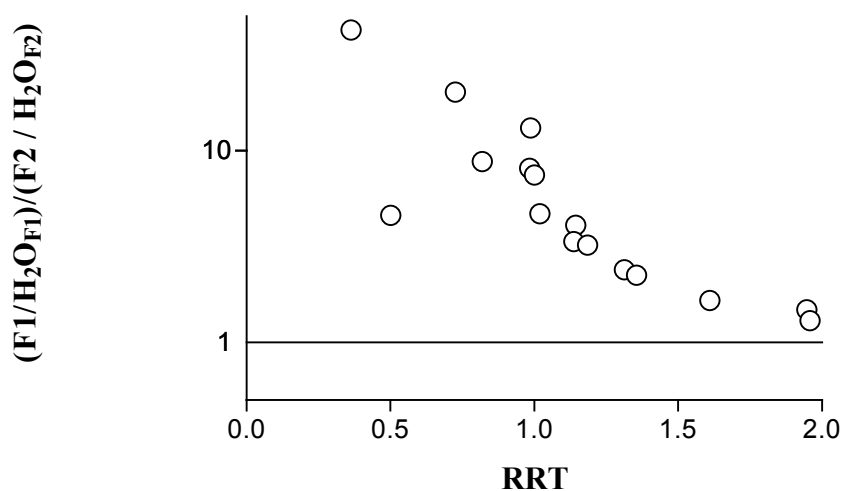


Figure 6: Plot of the relative retention times of organochlorines and PCDD/Fs versus the ratio of chemical co-distilled in F1 and F2 normalised to the respective water amount distilled (log 10 scale). Data from DVA2-9 with 30 g NaCl L⁻¹ and no TSS added.

An alternative way to analyse the results from the co-distillation study is to compare the fraction of chemical, as % of the total, that was distilled in F1 ($F1/(F1+F2+R)$) to the co-distilled fraction of the chemical in F2 (i.e. $F2/(F2+R)$). The data from DVA2-9, for example, indicate that co-distillation of those chemicals which do deplete relatively quickly is enhanced in F1 when the results are expressed on a volume basis (Figure 7). The reason for this behaviour is unclear at this stage but may suggest that some of the chemical are sorbed to the surface of the glass and the

lower relative co-distillation percentage in the second fraction is the result of kinetic limitation of desorption from the surface.

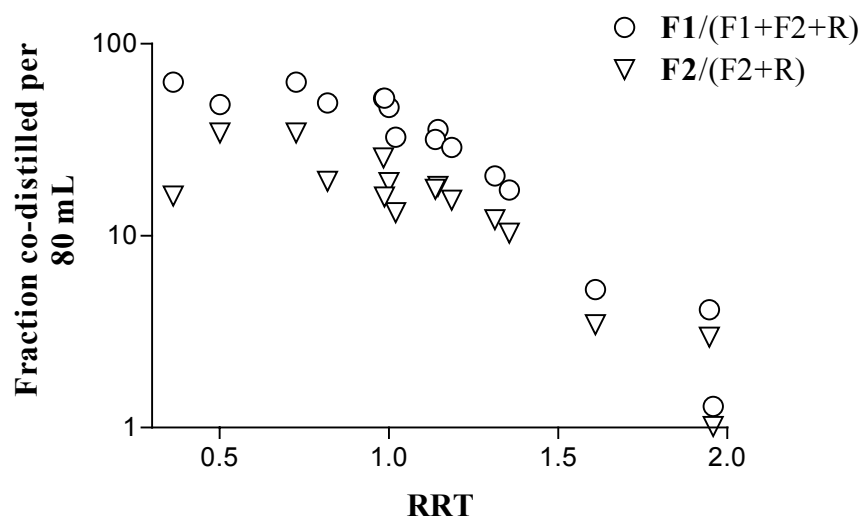


Figure 7: Plot of the relative retention times of organochlorines and PCDD/Fs versus the fraction of chemical co-distilled in F1 and F2 respectively – both normalised to a water volume of 80 mL (10 % of total water used in Expt 2). Data from DVA2-9 with 30 g NaCl L⁻¹ and no TSS added.

Despite these uncertainties it is noteworthy that an increase rather than a decrease in the co-distillation of chemicals with higher RRT was observable with an increase in salt concentration which is in agreement with the thermodynamics of mixtures and their colligative properties associated with increase in the boiling point of water.

Effect of Sorption Compartments in the Water on Co-distillation of Chemicals

To study the effect of extra sorption compartments in the water on the co-distillation of the chemicals of interest, a sample (DVA1-4) was spiked and equilibrated with a known concentration of total suspended sediments (TSS) (0.2 g L⁻¹) and 20 g NaCl L⁻¹. Inclusion of a new sorption compartment in the system resulted in a decrease in co-distillation compared with samples DVA1-1 – DVA-3. However the results from DVA1-4 with a 0.2 g TSS L⁻¹ sorption compartment showed that significant quantities of the individual compounds can co-distill (see Figure 8).

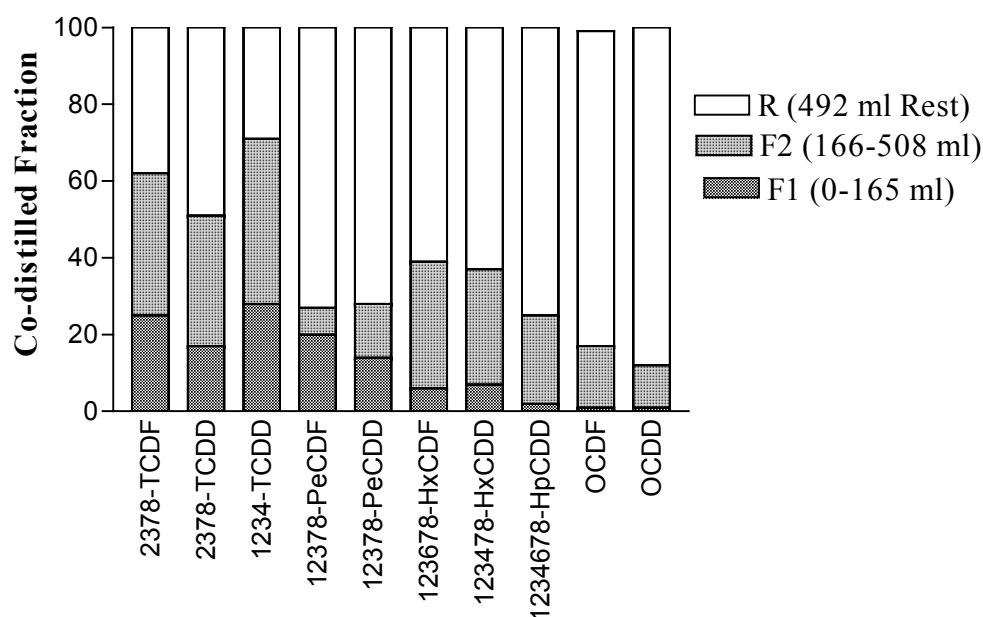


Figure 8: Quantity of PCDD/F congeners co-distilled from water containing 0.2 g total suspended sediment.

The results of the first experiment provided the first indication that suspended solids in the water will affect co-distillation. The main focus of Experiment 2 was to investigate the effect of TSS in the water on the co-distillation of hydrophobic organic chemicals. For the purpose of Experiment 2 water samples were spiked with total suspended sediment to give 0, 0.16, 0.48 and 1.44 g TSS L⁻¹. Then, in accordance with Experiment 1, samples were equilibrated for at least 2 weeks and about 10 % (F1) and an additional 30 % (F2) fractions of the water were distilled from the samples as described above.

Again, in all treatments, significant co-distillation was observed. However, the data indicated a trend of decreasing co-distillation with an increase in the TSS (Figures 9). For example, the co-distillation of 2,3,7,8-TCDD with F1 ranged from about 90 % in one of the control treatments to which no TSS was add to about 38 % in the sample with 1.44 g TSS L⁻¹. Furthermore the results suggest that the effect of TSS on co-distillation is compound-specific with a trend of decreasing effect of TSS on co-distillation efficiency (i.e. the slope of the curve decreases) with increasing compound

RRT (Figure 10). However, the linear regression models using the data set in Appendix I, Table 2, which have been obtained from the linear regression lines demonstrate that if any difference in effect between compounds is observable, the effect of TSS on a decrease in co-distillation is greatest for the higher molar mass chemicals such as OCDD or TCDD (i.e. the linear regression models in Table 2, Appendix I, predict that for DDTs or tetra- and pentachlorinated PCDD/Fs an increase in TSS concentration from 0.5 to 1.5 g L⁻¹ will result in a decrease in co-distillation in F1 by about 30-50 % compared to 55 – 70 % decrease for hepta- and octachlorinated PCDD/Fs).

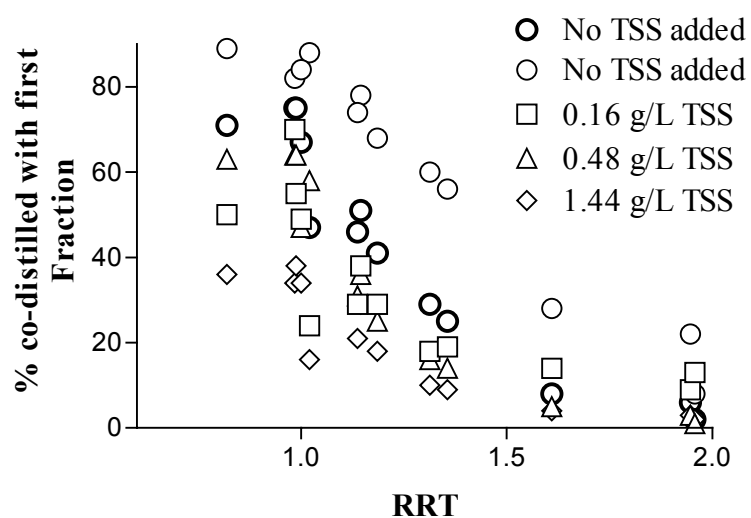


Figure 9: Plot of the relative retention times of organochlorines and PCDD/Fs versus the fraction of chemical co-distilled in F1 at different amounts of total suspended sediments (TSS). Data from DVA2-5 - DVA2-9

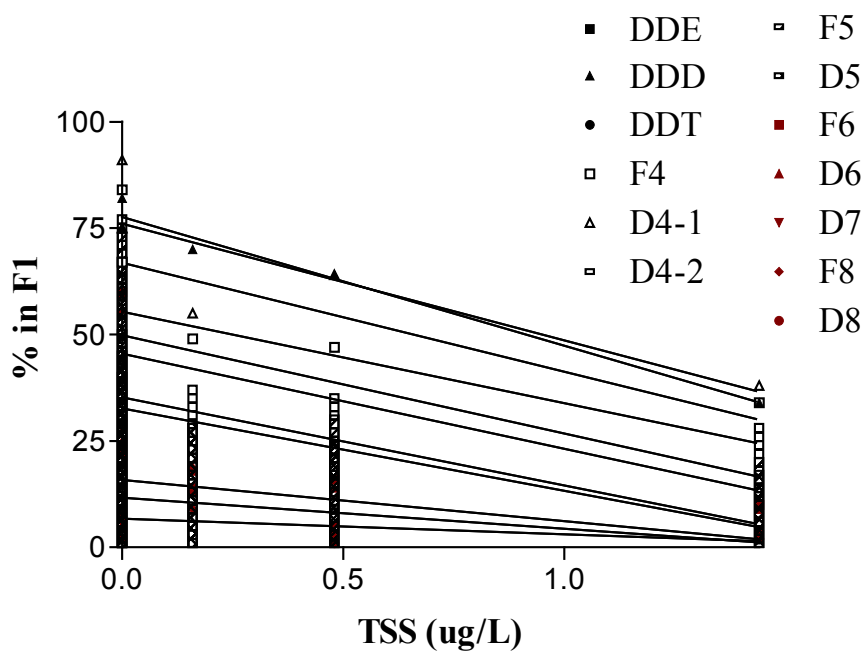


Figure 10: Co-distillation of DDTs and PCDD/Fs from natural estuarine water as a function of total suspended sediments. Data from DVA2-5 - DVA2-9.

At this point co-distillation of organochlorines and PCDD/Fs was investigated in samples which were prepared in the laboratory from pure water and chemicals. To finally confirm these effects, a comparison of these results was made with estuarine water samples. Water was collected from the Brisbane River and a known volume of this water was added to a known quantity of organochlorines and PCDD/Fs, mixed and equilibrated for 4 weeks on a shaker in the laboratory (Note that a blank of the river water was also analysed, but no chemicals of interest were detectable). Again co-distillation of organochlorines and PCDD/Fs was clearly observable in both samples. Between 48 and 62 % of the 2,3,7,8-TCDD was co-distilled with the first 10 % of the distilled water (Figure 11).

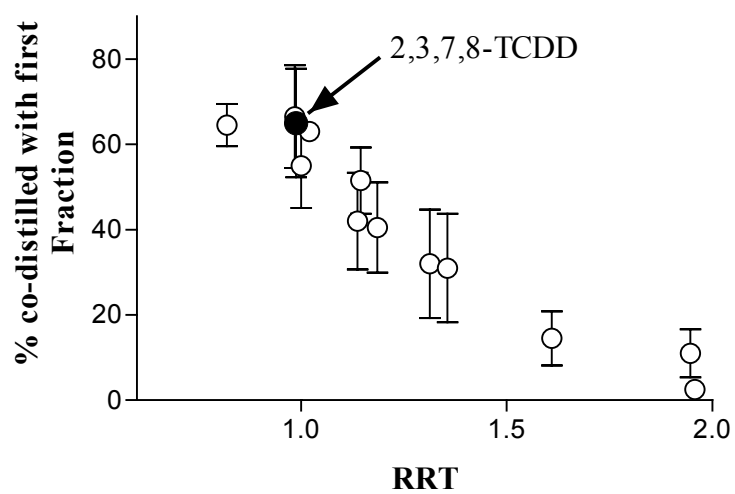


Figure 11: Plot of the relative retention times of organochlorines and PCDD/Fs versus the mean percentage of co-distilled chemicals observed in F1 using water samples collected from the Brisbane River Estuary.

Phase II – Evaluation of Co-distillation of Dimethylarsenic Acid – Agent Blue.

Background to Agent Blue – dimethyl arsenic acid (DMA)

Properties and environmental fate of DMA: In phase 2 of the project the focus was to evaluate co-distillation of Agent Blue, better known as dimethylarsenic acid (DMA) or cacodylic acid. DMA has been and is still used as a contact herbicide. It is still used in some countries such as the U.S.A. as a defoliant in cotton cultivation but also for weed control in areas which are not used for crop production (i.e. around buildings, fences, and in forest management).

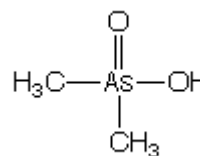
Compound Properties

DMA - C₂ H₇ AS O₂ (62) MW: 138.0

Colorless crystals (pure compound). The technical grade is 65% pure, sodium chloride being one impurity (62).

MELTING POINT: 192-198°C (pure compound) (62); 200°C (pure chemical)

SOLUBILITY: 2 kg/kg water at 25°C (pure compound) (62).



Dimethyl arsenic acid

DMA is a polar compound, highly soluble in water but practically insoluble in organic solvents. It is a clear yellowish-tan liquid. In Vietnam about 4.4 million liters (1.12 Mil.Gallons) of Agent Blue were used between 1962-1971. Interestingly, Agent Blue was used for different purposes than Agent Orange. About 50 % of the Agent Blue was used for crop destruction missions where it was the agent of choice for the destruction of rice crops. The remaining 50 % of Agent Blue was used around base perimeters and was applied by helicopters or ground vehicles (Young et al. 1978 quoted in IoM, 1999). Agent Blue is most effective against grassy plants and acts by absorbing moisture from leaves (desiccation). The application of Agent Blue is usually in the range of 4.5 – 9 litres per acre; for tall grasses, much higher doses were used because of the large foliage masses. According to the pesticide manual DMA is quickly adsorbed on soil particles and ion exchange sites and thus inactivated upon soil contact. Microbial degradation in the soil is considered to be the key process for loss of DMA from the soil with 15-80% of DMA being lost in 8 months. On the other

hand, DMA is practically non-volatile and degradation through UV radiation is not a relevant loss process.

Toxicology of DMA:

The authors of this study are not experts on the toxicity of DMA and therefore asked Dr Jack Ng, the leader of the Metals and Metalloids Toxicity program at ENTOX and an expert on arsenic toxicity, to provide a review of DMA and arsenic toxicity to this report. This review is beyond the scope of this report and therefore provided as an appendix (Appendix II). The report is provided here but remains the intellectual property of Dr Ng.

Result of the DMA Distillation Experiment

In this study we investigated whether in addition to Agent Orange contaminants, Agent Blue may also co-distil and thus drinking water contaminated with DMA may be a relevant exposure pathway. For the purpose of this study DMA (approximately 1 µg) was placed in a flask and RO water added. The solution was equilibrated as previously. Three distilled fractions (10 %, 10-30% and 30-90%) as well as the non-distilled fractions were analysed using ICPMS.

No DMA or only trace levels of DMA, detectable as arsenic, was found in any of the distilled fractions. In contrast the non-distilled fractions required dilution prior to analysis since they contained the now concentrated DMA. Since DMA is a very polar compound with extremely low vapour pressure and high water solubility this result was in agreement with the working hypothesis that DMA should not co-distill. This result suggests that DMA is unlikely to have been consumed in substantial quantities through drinking water that was produced by distillation.

A further issue is that DMA, unlike Agent Orange, was apparently used primarily for crop destruction and around army bases and not for the defoliation of mangrove areas. Hence at this point it seems less likely that DMA from the spraying of Agent Blue contributed significantly to the direct exposure of RAN members on board ships. An exception, however, may be the potential contamination of food (and in particular fish) and fresh water that was obtained from Vietnam. At this point some uncertainty

persists to whether food was obtained in significant quantities from Vietnam for supply on board navy ships.

Formation Study

Formation of PCDD/Fs from Agent Orange components could not be measured in the water with the present experimental set-up. This aspect of the experimental studies was not fully satisfactory. The 2,4,5-T used for the experiment was contaminated with PCDDs which complicates the interpretation of the results (i.e. we would have to evaluate an increase of TCDD concentration which is much more difficult than the appearance of a practically non-existent compound). Secondly, there were problems with determining suitable spiking volumes of Agent Orange components as well as with the standard solutions for elution. Finally, a key problem of this section of the study was that the temperature of the surface of the copper in the distillation unit on board ship was probably about 110 °C while the copper element in the laboratory experiments were set to heat the water to 60 °C (which is equivalent to the water temperature in the distillation units on ships). While we can draw few conclusions from the formation experiments to date we aim to set-up new experiments in near future in which we hope to resolve most of the problems that disallow any valid conclusion from this part of the study.

Preliminary Estimation of Dioxin Exposure Uptake by Sailors from Water

A key goal of this study was to attempt an evaluation of exposure of the RAN personnel aboard ship on duty in Vietnam by consumption of contaminated water as well as from other potential pathways. The following assessment should only be seen as a preliminary attempt as still many of the input factors remain uncertain. Firstly, we have attempted to estimate exposure directly through the consumption of distilled water. Due to the lack of information on water concentrations of PSDD/Fs from the period and at sites of interest, we attempted to calculate water concentrations from concentrations in fish from Vietnam waters taken during the relevant period (Baughman and Meselson, 1973).

The TCDD concentration in fish described by Baughman and Meselson (1973), gives an estimate of the fish lipid concentration (2.5 %) and experimentally determined fish-water bioconcentration factors are available (Loonen et al. 1994). A best estimate suggests that the TCDD water concentration in the Vietnam waters where Baughman and Meselson 's fish were exposed were in the range of 0.04 – 0.7 ng/L.

The distillation experiments carried out in the present study show that, if we assume that about 50 % of the water is distilled and the rest is discharged, distillation would result in an enrichment of TCDD by about a factor of 2 (i.e. 0.08 – 1.4 ng/L). Recent information suggests that possibly only 5-10 % of the water was distilled which would increase this enrichment factor to approximately 10-20, depending on the TSP of the water.

We assumed that on average sailors consumed on average 5 L of this water per day. The direct consumption of this water would lead to a daily body burden of about 0.4 – 7 ng/day. The water was also used to prepare food and as a result of the hydrophobic character of the dioxins the TCDD would also accumulate in the food. Hence we may estimate that the total exposure due to water contaminating food was similar to that of the direct consumption of drinking water – another 0.4 – 7 ng/day.

Table 2: Exposure Model Input Parameter including uncertainty.

		Literature values used	Estimated Uncertainty	Reference
A	Concentration of TCDD in fish	49 – 1020 ng/kg FW	Large > 10	Baughman & Meselson, 1973
B	Estimated fish lipid content to calculate fish lipid based concentration	2.5 %	2-4	Mueller et al. 1997
C	Estimated fish lipid based concentration $C=A*100/B$	2.8 – 44 ug/kg lipid	See col. A & B	
D	Bioconcentration factor (fish lipid- water)	81000-170000	Medium – less 5 – 10	Loonen et al. 1994
E	Calculated water concentration upper estimate using $E=C/D^s$	0.043 – 0.69 ng / L water	Large > 10	
F	Enrichment factor from distillation assuming 50 % water is distilled	2 [#]	Low (<2)	This study
G	Period for which water was distilled during period in Vietnam Waters	14 days	Low (<5)	Personal Communication RAN
H	Daily portion of water directly consumed by RAN members	5 L	Low (<2)	Personal Communication RAN

^sNote that the density of the fish lipid was assumed to be 1 kg/L thus the values in C are directly transformed to a per L lipid basis.

[#]Likely to be substantially underestimated according to recently acquired information. Probably 10 is more appropriate.

Now it is important to estimate the overall exposure period. According to reports from RAN personnel the rule on board ship was to produce drinking water primarily during the periods when the ship was in the turbid estuarine water since the water was less pure and could have caused potential damage to the engines if used in boilers. While in the pristine water offshore the distillation units produced water primarily for the ships engine. Hence the drinking water that was produced during the periods in contaminated waters lasted for a significant portion of the return trip. If we assume that a 14 days reserve was produced during such a trip, the total body burden of dioxin through direct consumption of water that originated from distilling in Vietnamese waters are estimated to be between about 10 ng to 190 ng per journey. For a 70 kg person the body burden would be in the range of 12 - 200 pg / kg bw per day for a 14 day period in addition to the background exposure through TCDD and other dioxin-like chemicals in the food and the environment (It should be noted that the late 1960s and the early 1970s were probably the peak exposure periods for these chemicals and the body burden of the population was thus already substantially higher than at present).

The uncertainty of these exposure assessment figures is relatively large. The key uncertainties are related to the concentration in the fish and the calculation of the water concentration from these fish data. Since the anchorage of the Australian RAN ships near Van Tau were in the vicinity of a large mangrove region that was regularly sprayed with Agent Orange, it is likely that the water concentrations at the anchorage were higher than those predicted from the fish data.

Comparison of Exposure Data with Effects

While it is beyond the scope of this study to evaluate effects of dioxin exposure in RAN members it is of interest to put the exposure data into some perspective relative to current guidelines and observable effect values. At present (2002) a series of intake criteria are applied by WHO and various committees in Europe and the key difference in the guidelines are the exposure period over which the intake value is calculated. In principle the WHO has targeted a daily intake of 2 pg TEq/kg bw per day whereas other European committees have expressed their values on weekly exposure (14 pg kg bw per week) or a monthly value (70 pg kg bw per month). In

terms of risk assessment it is reassuring that various committees developed very similar values despite following different methods for establishing these intake values. On the other hand these guidelines are all based on a similar range of toxicological studies where animal studies and biochemical markers were used to obtain low observable effect levels for the various endpoints (van Leuwen 2001, oral presentation at NRCET) (Table 3). Australia's NHMRC has recently distributed a document in which the value of 70 pg per kg bw per month was recommended for adoption in Australia.

A notable exception to these agreements in international guidelines for dioxin exposure in humans is the approach taken by the US-EPA who established a recommended guideline which is more than 2 orders of magnitude lower than the values set by the WHO/ICPS and various European committees. The US-EPA have concluded in their recent dioxin-reassessment document that the dioxin background contamination which is in the range of 0.5 – 2 pg per kg bw per day at the present may pose a significant cancer risk between 10^{-2} – 10^{-3} .

In comparison, the exposure of the RAN members from water related sources may have been 12-200 pg /kg bw /day or 360 – 6000 pg / kg bw / month. This indicates that in addition to normal background exposure, RAN members may have received exposure which is one to two orders of magnitude above the acceptable intake values and at a level above the observed effect levels in experimental animals (Table 3).

Table 3: Animal body burdens and related estimated human daily intake (EHDI)
(Table from van Leeuwen, presentation at NRCET, August 2001)

Response	Maternal body burden (ng/kg bw)	Human EDI (pg/kg bw 1 day)
Rats		
Decreased sperm counts	28	14
Immune suppression	50	25
Genital malformations	73	37
Monkeys		
Neurobehavioural effects	42	21
Endometriosis	69	35

Table 4: Acceptable intake guidelines of dioxin-like chemicals. Comparison between the standards for human intake of dioxins, furans and dioxin-like PCBs agreed by WHO (1998), EC-SCF (2001) and FAO/WHO (2001).

Standard	pg / kg bw / day	pg / kg bw / week	pg / kg bw / month
WHO consultation (1998)	1-4	(7-28)	(30- 120)
EC-SCF (2001)	(2)	14	(60)
FAO/WHO (2001)	(2.3)	(16)	70

Exposure values in brackets were calculated for the other periods for easier comparison.

CONCLUSION

This study evaluated the potential for co-distillation of organic pollutants by ship distillation units.

This study clearly demonstrated that distillation of water contaminated with hydrophobic chemicals such as dioxin-like chemicals of organochlorine pesticides results in contamination of the distilled water. The extent of co-distillation largely depends on the physico-chemical properties of the compounds of interest. 2,3,7,8-Tetrachlorodibenzodioxin, the primary contaminant in Agent Orange, the defoliant used in the Vietnam conflict, co-distills relatively rapidly and thus will be enriched in the distilled water. Other PCDD/Fs with up to six chlorine substitutions as well as all organochlorine chemicals investigated also have the tendency to enrich in the distilled water when compared to the source water.

A relationship between physico-chemical properties and co-distillation is apparent since with increasing relative retention time the tendency for co-distillation decreased. The effect of salinity on co-distillation could not be determined, but the studies indicated that suspended particles or a sorption compartment in the water reduced the tendency for co-distillation. However, enrichment of TCDD in the distilled water was still high at 1.4 g TSS L⁻¹. The same was found for water which originated from the Brisbane River Estuary which suggests that co-distillation of such chemicals is highly likely.

During this study, it was noted that evaporative distillation is widely used through the world. Based on the results of this study, the authors strongly recommend that for a prevention of contamination of all who consume water produced by evaporative distillation it should be made certain that the source water used to be distilled does not contain volatile or semivolatile hydrophobic pollutants. If this cannot be guaranteed we recommend the use of a post-distillation purification step such as a sorption phase that removes such contaminants. (Activated charcoal or carbon filters would be suitable for this purpose.)

REFERENCES

- AIHW – Australian Institute of Health and Welfare. 1999. *Morbidity of Vietnam veterans: A study of the health of Australia's veteran community. Vol 3: Validation Study*. AIHW, Canberra.
- Baughman, R., and M. Meselson. 1973. An analytical method for detecting TCDD (Dioxin): Levels of TCDD in samples from Vietnam. *Environmental Health Perspective* 5: 27-35.
- Beck, H, K Eckart, W Mathar, and R Wittkowski. 1989. PCDD and PCDF body burden from food intake in the Federal Republic of Germany. *Chemosphere* 18, no. 1-6: 417-24.
- Cavanagh, JE. 2000. *Organochlorine Insecticide Usage in the Sugar Industry of the Herbert and Burdekin River Regions: chemical, biological, and risk assessments*. Unpublished thesis, James Cook University, Australia.
- Crane, PJ, DL Barnard, KW Horsley and MA Adena. 1997a. *Mortality of Vietnam Veterans: the veteran cohort study. A report of the 1996 retrospective cohort study of Australian Vietnam veterans*. Department of Veterans' Affairs, Canberra. ISBN 0 642 27098 8
- Crane, PJ, DL Barnard KW Horsley and MA Adena. 1997b. *Mortality of National Service Vietnam Veterans. A report of the 1996 retrospective cohort study of Australian Vietnam veterans*. Department of Veterans' Affairs, Canberra. ISBN 0 642 38363 X.
- EC-SCF – EC Health and Consumer Protection Directorate-General. 2001. *Opinion of the Scientific Committee on Food on the Risk Assessment of Dioxins and Dioxin-like PCBs in Food*. (Update based on new scientific information available since the adoption of the SCF Opinion of 22nd November 2002). European Commission, Brussels, Belgium, Document CS/CNTM/DIOXIN/20 final. Adopted on 30 May 2001.
- FAO/WHO. 2001. 57th JECFA Meeting, Rome, 5-14 June 2001: Summary and Conclusions, Annex 4, pp24-40.

Watervolatility of PCDD/Fs

- Fürst, P. 1998. Dioxine in Lebensmittel. *Handbuch Dioxine*. 1st ed., Ed. M. Oehme, 227-66. Heidelberg: Spektrum Akademischer Verlag.
- Hülster, A. 1994. *Transfer von polychlorierten Dibenzo-p-dioxinen und Dibenzofuranen (PCDD/PCDF) aus unterschiedlich stark belasteten Böden in Nahrungs-und Futterpflanzen*. Verlag Ulrich E. Grauer, Germany.
- IARC – International Agency for Research on Cancer. 1997. *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans Vol 69. Polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans*. World Health Organisation, France.
- IoM – Institute of Medicine 1999. *Veterans and Agent Orange, Update 1998*. Washington DC, USA, National Academy Press.
- Hattemer-Frey, H. A., and Travis C.C. 1989. Comparison of human exposure to dioxin from municipal waste incineration and background environmental contamination. *Chemosphere* 18, no. 1643-649.
- Liem, D. A. K., P. Fuerst, and C. Rappe. 2000. Exposure of populations to dioxins and related compounds. *Food Additives and Contaminants* 17, no. 4: 241-59.
- Loonen, H, JR Parsons and HAJ Govers. 1994. Effect of sediment on the bioaccumulation of a complex mixture of polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) by fish. *Chemosphere* 28, no. 8: 1433-1446.
- Lyman, WJ, WF Reehl and DH Rosenblatt. 1990. *Handbook of chemical property estimation methods*. Washington DC USA: American Chemical Society.
- Mackay, D, W Shiu, and KN Ma. 1992. *Illustrated handbook of physical-chemical properties and environmental fate for organic chemicals Vol 1. Monoaromatic hydrocarbons chlorobenzenes and PCBs*, Lewis Publisher, Chelsea, Mi, USA.
- McLachlan, MS. 1997. A simple model to predict accumulation of PCDD/Fs in an agricultural food chain. *Chemosphere* 34, no. 5-7: 1263-76.

- McLachlan, MS. 1996. Biological uptake and transfer of polychlorinated dibenzo-p-dioxins and dibenzofurans. *Issues in Environmental Science and Technology*. Number 6 ed., R. E. Hester, and R M Harrison, 31-52. Vol. 6. Cambridge UK: The Royal Society of Chemistry.
- Müller, JF. 1997. *Occurrence and Distribution Processes of Semivolatile Organic Chemicals in the Atmosphere and Leaves*. Unpublished thesis, Griffith University, Australia.
- Müller, JF, MS McLachlan, DW Hawker, and DW Connell. 1997. Bioconcentration of persistent semivolatile hydrophobic chemicals in the vegetation/atmosphere system. In: *Ecological Issues and Environmental Impact Assessment*. Ed. Cheremisinoff P.N., 421-42. Houston, Texas, USA: Gulf Publishing.
- Naval Marine Engineering Practice, Vol 1. 1959. *Ch 12: Auxiliary Machinery*. Her Majesty's Stationery Office, London.
- Paasivirta, J, S Sinkkonen, P Mikkelsen, T Rantio, F Wania. 1999. Estimation of vapor solubilities and Henry's law constants of selected persistent organic pollutants as functions of temperature. *Chemosphere* 39, no. 5: 811-832.
- van Leuwen RFX. August 2000. Health Risk Assessment of dioxins. Oral presentation at the National Research Center for Environmental Toxicology (NRCET), Queensland, Australia.
- Windholz, M (Ed). 1983. *The Merck Index: An encyclopedia of chemicals, drugs and biologicals*. Merck & Co. Inc, United States of America.
- WHO – World Health Organisation. 1998. Assessment of the health risks of dioxins: re-evaluation of the tolerable daily intake (TDI). *Executive Summary of the WHO Consultation*, May 25-29, Geneva, Switzerland.

APPENDIX I

Watervolatility of PCDD/Fs

Appendix Table 1: Relative quantity of organochlorines and PCDD/Fs. Blue shaded areas mark uncertainty with peaks due to interferences and broad peaks, yellow shaded areas – sample not quantifiable and result based on average of total in other samples – (F2+R)

	Blank	DVA1-1 0 NaCl; 0 TSS		DVA1-2 15 g/L NaCl; 0 TSS		DVA1-3 30 g/L NaCl; 0 TSS			DVA1-4 30 g/L NaCl; 0.2 g/L TSS		
		F1	R	F1	R	F1	F2	R	F1	F2	R
Distilled amount		141	629	92	620	161	343	496	165	343	492
HCB	ND	114	ND	100	0	91	9	0	NA	NA	NA
Lindane	ND	93	ND	100	0	69	31	0	NA	NA	NA
Heptachlor	ND	101	ND	100	0	100	0	0	NA	NA	NA
Aldrin	ND	82	3	100	0	100	0	0	NA	NA	NA
Heptachlorepo.	ND	102	1	100	0	94	6	0	NA	NA	NA
DDE	ND	81	ND	96	4	87	11	2	NA	NA	NA
dieldrin	ND	88	ND	100	0	100	0	0	NA	NA	NA
DDD	ND	85	ND	100	0	83	16	1	NA	NA	NA
DDT	ND	73	ND	100	0	85	14	1	NA	NA	NA
2,3,7,8-TCDF	ND	79	5	79	21	84	11	5	25	37	38
2,3,7,8-TCDD	ND	85	3	95	5	87	9	3	17	34	49
1,2,3,4-TCDD	ND	51	10	65	35	82	11	7	28	43	29
1,2,3,7,8-PeCDF*	ND	46	15	48	52	80	0	20	20	7	73
1,2,3,7,8-PeCDD*	ND	42	NQ	40	60	84	0	16	14	14	72
1,2,3,6,7,8-HxCDF	ND	40	NQ	24	76	65	14	21	6	33	61
1,2,3,4,7,8-HxCDD	ND	34	NQ	18	82	57	17	26	7	30	63
1,2,3,4,6,7,8-HpCDD	ND	20	NQ	6	94	45	18	37	2	23	75
OCDF	ND	16	NQ	3	97	42	12	47	1	16	82
OCDD	ND	13	NQ	1	99	40	6	54	1	11	88

Watervolatility of PCDD/Fs

Experiment 2

	DVA2-3 30 g/L NaCl; 0 TSS			DVA2-4 Brisbane River			DVA2-5 Brisbane River			DVA2-6 0.2 g TSS L ⁻¹ ; 30 g NaCl		
	F1	F2	R	F1	F2	R	F1	F2	R	F1	F2	R
Distilled amount	78	231	491	64	233	503	84	239	509	84		492
HCB												
Lindane												
Heptachlor												
Aldrin												
Heptachlorepo.				46	54	0						
DDE	89	9	2	61	29	11	68	13	19	50	27	22
dieldrin												
DDD	82	17	1	58	38	4	75	21	4	70	30	0
DDT	88	11	2	62	38	0	64	21	15	24	35	41
2,3,7,8-TCDF	84	9	7	48	36	16	62	18	20	49	29	22
2,3,7,8-TCDD	91	7	3	56	34	10	74	14	12	55	32	13
1,2,3,4-TCDD	78	10	12	46	36	18	57	15	27	38	28	34
1,2,3,7,8-PeCDF*	74	13	13	34	40	26	50	18	33	29	33	38
1,2,3,7,8-PeCDD*	68	15	17	33	40	27	48	16	35	29	30	41
1,2,3,6,7,8-HxCDF	60	17	22	23	36	41	41	13	46	18	26	56
1,2,3,4,7,8-HxCDD	56	18	26	22	35	43	40	12	48	19	25	56
1,2,3,4,6,7,8-HpCDD	28	14	58	10	19	70	19	4	77	14	11	75
OCDF	22	14	64	7	11	82	15	3	82	9	6	84
OCDD	8	7	85	3	5	92	2	2	96	13	4	84

Shaded columns – due to interferences no quantification could be performed in this sample – the % are calculated assuming the mean recovery from DVA2-3 and DVA-4.

Watervolatility of PCDD/Fs

	DVA2-7 30 g/L NaCl; 0.60 g TSS L ⁻¹			DVA2-8 30 g/L NaCl; 1.8 g TSS L ⁻¹			DVA2-9 30 g/L NaCl; 0 g TSS L ⁻¹		
	F1	F2	R	F1	F2	R	F1	F2	R
Distilled amount	86	219	495	75	238	487	115	233	452
HCB									
Lindane									
Heptachlor									
Aldrin									
Heptachlorepoxy.									
DDE	63	26	11	36	36	29	71	16	13
dieldrin									
DDD	64	29	7	34	42	24	75	19	7
DDT	58	31	12	16	46	38	47	20	33
2,3,7,8-TCDF	47	33	20	34	31	35	67	18	15
2,3,7,8-TCDD	64	24	12	38	36	26	75	12	13
1,2,3,4-TCDD	36	33	31	29	29	43	51	26	23
1,2,3,7,8-PeCDF*	31	33	36	21	26	54	46	28	27
1,2,3,7,8-PeCDD*	25	34	41	18	24	58	41	26	32
1,2,3,6,7,8-HxCDF	16	26	58	10	14	76	29	25	46
1,2,3,4,7,8-HxCDD	14	28	58	9	16	74	25	23	53
1,2,3,4,6,7,8-HpCDD	5	13	82	4	9	87	8	9	83
OCDF	3	7	90	3	7	91	6	8	86
OCDD	1	5	94	2	5	93	2	3	95

Appendix I Table 2: Parameters from linear regression between the relative fraction of chemicals codistilled in F1 (%) and the amount of total suspended sediments in the treatment. In all cases $n = 5$ and besides two controls treatments included 0.16; 0.48 and 1.44 g TSS L⁻¹.

Compound	slope	intercept	r ²	p
DDE	-21	73	0.62	0.11
DDD*	-24	76	0.97	0.0017
DDT	-23	59	0.37	0.28
2,3,7,8-TCDD	-22	76	0.69	0.084
1,2,3,4-TCDD	-17	55	0.45	0.22
1,2,3,4,7-PeCDD	-18	46	0.47	0.20
1,2,3,4,7,8-HxCDD	-16	33	0.40	0.25
1,2,3,4,6,7,8-HpCDD	-7.8	16	0.35	0.29
OCDD	-2.9	6.7	0.18	0.47
2,3,7,8-TCDF	-21	67	0.63	0.11
2,3,4,7,8-PeCDF	-19	50	0.45	0.22
1,2,3,4,7,8-HxCDF	-17	35	0.40	0.25
OCDF	-5.8	12	0.31	0.33

Appendix I Table 3: Experimental set-up for distillation experiments including quantity of water distilled with each fraction (in mL)

	F1	F2	Rest
Experiment 1 – Total Volume 1000 mL			
Expt 1: (DVA-1 0- B0) RO Blank	Combined		
Expt 1: (DVA-1-B11) RO – Blank	107	893 (Combined)	
Expt 1: (DVA-1-B12) RO + TSS Blank	Combined		
Expt 1: (DVA-1-V1) RO + SPIKE	141	230	629
Expt 1: (DVA-1-V2) RO + 15 g NaCl + Spike	92	288	620
Expt 1: (DVA-1-V3) RO + 30 g NaCl + Spike	161	343	496
Expt 1: (DVA-1-V4) RO + 30 g NaCl + 0.2 g TSS +SPIKE	165	259	575
Expt 1: (DVA-1-R) RO + 30 g NaCl + 0.2 g TSS +SPIKE	Combined for recovery		
Experiment 2 – Total Volume 800 mL			
Expt.2 (DVA-2-1) RO + 24 g NaCl – BLANK	70	280	450
Expt.2 (DVA-2-2) Brisbane River – BLANK	65	230	505
Expt.2 (DVA-2-3) (RO+24 g NaCl+SPIKE)	78	231	491
Expt.2 (DVA-2-4) (Brisbane River + SPIKE)	64	233	503
Expt.2 (DVA-2-5) (Brisbane River + SPIKE)	84	239	477
Expt.2 (DVA-2-6) (RO+24 g NaCl+ 0.16 g TSS + SPIKE)	86	225	489
Expt.2 (DVA-2-7) (RO+24 g NaCl+ 0.48 g TSS + SPIKE)	86	219	495
Expt.2 (DVA-2-8) (RO+24 g NaCl+ 1.44 g TSS + SPIKE)	75	238	487
Expt.2 (DVA-2-9) (RO+24 g NaCl+ 0 g TSS + SPIKE)	115	233	348

Appendix I Table 4: Summary of Experimental variations

	Preliminary Expt	Experiment 1	Experiment 2
Water used	RO-Water	RO-Water	RO-Water Brisbane River Water
Salinity	0 g NaCl L ⁻¹	0 g NaCl L ⁻¹ 15 g NaCl L ⁻¹ 30 g NaCl L ⁻¹	0 g NaCl L ⁻¹ 30 g NaCl L ⁻¹
Total suspended solids	0 g L ⁻¹	0 mg L ⁻¹ 200 mg L ⁻¹	0 mg L ⁻¹ 200 mg L ⁻¹ 600 mg L ⁻¹ 1800 mg L ⁻¹
Compounds used	OC Pesticides	Dioxins and OC pesticides	Dioxins and OC pesticides
Clean-up	None	None	Minicolumn
Instrumental Analysis	GC-ECD	GC-ECD and GC-MS	GC-MS

Appendix I Table 5: Variations of the experimental set-up for the formation study.

Variants	1-liter dest. Water with 30g NaCl	1-liter Brisbane-River water
Blank	+ 20µl Spike Mix* + 15µl Recovery Standard + Warm up to 60°C with copper header (8h)	+ 20µl Spike Mix* + 15µl Recovery Standard + Warm up to 60°C with copper header (8h)
2,4-D	+ 20µl Spike Mix* + 15µl Recovery Standard + 1µg 2,4-D + Warm up to 60°C with copper header (8h)	+ 20µl Spike Mix* + 15µl Recovery Standard + 1µg 2,4-D + Warm up to 60°C with copper header (8h)
2,4-D Control	+ 20µl Spike Mix* + 15µl Recovery Standard + 1µg 2,4-D + Stay for 8h without heading	+ 20µl Spike Mix* + 15µl Recovery Standard + 1µg 2,4-D + Stay for 8h without heading
2,4,5-T	+ 20µl Spike Mix* + 15µl Recovery Standard + 1µg 2,4,5-T + Warm up to 60°C with copper header (8h)	+ 20µl Spike Mix* + 15µl Recovery Standard + 1µg 2,4,5-T + Warm up to 60°C with copper header (8h)
2,4,5-T Control	+ 20µl Spike Mix* + 15µl Recovery Standard + 1µg 2,4,5-T + Stay for 8h without heading	+ 20µl Spike Mix* + 15µl Recovery Standard + 1µg 2,4,5-T + Stay for 8h without heading
2,4-D and 2,4,5-T	+ 20µl Spike Mix* + 15µl Recovery Standard + 1µg 2,4-D + 1µg 2,4,5-T + Warm up to 60°C with copper header (8h)	+ 20µl Spike Mix* + 15µl Recovery Standard + 1µg 2,4-D + 1µg 2,4,5-T + Warm up to 60°C with copper header (8h)
2,4-D and 2,4,5-T Control	+ 20µl Spike Mix* + 15µl Recovery Standard + 1µg 2,4-D + 1µg 2,4,5-T + Stay for 8h without heading	+ 20µl Spike Mix* + 15µl Recovery Standard + 1µg 2,4-D + 1µg 2,4,5-T + Stay for 8h without heading

*Spike Mix = 50% Internal Standard Solution and
50 %Supplement Internal Standard/Solution

APPENDIX II:

Appendix II A REVIEW OF ARSENIC TOXICITY

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Sources of arsenic in the environment

Arsenic is an element that occurs widely in rocks, soil, water, air, plants, and animals. Arsenic ranks 20th in abundance in the earth's crust (Cullen et al., 1989). Arsenic is a ubiquitous element. The crust of the earth contains about 2mg/kg of arsenic and is highest at 620mg/kg in its deep inside core (Zheng, 1994). Arsenic is a metalloid exhibiting chemical and physical properties of both metallic and non-metallic nature. There are three kinds of arsenic derivatives with the color of grey, yellow and black respectively. Grey arsenic is also called metallic arsenic and is both a fine thermo-conductor and electro-conductor and relatively stable at normal temperatures (Wang, 1997). Arsenic can show either electro-positive or electro-negative valence in its compounds. The valencies of arsenic are 0, -3, 3 and 5. Although arsenic is found in nature to a small extent in its elemental form, it occurs mostly as inorganic and organic compounds in either the As^{(III)(+3)} or As^{(V)(+5)} valency state, as in AsF₃ and AsF₅ (Welch et al., 1988). Arsenic gains three electrons and forms AsH₃ (Arsine) when reacted with hydrogen, but loses three or five electrons in forming As₂O₃ or As₂O₅. These two forms of arsenic are inorganic species and are the forms most prevalent in water (Irgolic, 1994; Clifford et al., 1994; USEPA, 2000).

There are numerous natural sources as well as anthropogenic activities that may introduce arsenic into food and drinking water. The primary natural sources of arsenic include geological formations such as rocks, soil, and sedimentary deposits, geothermal activity, and volcanic activity. These contribute to the regional background base level of arsenic and to abnormal geochemical arsenic conditions in some local areas. The major present and past sources of arsenic distribution influenced by human activities include the use of arsenic in wood preservatives, agricultural uses (including the use as pesticides), industrial uses, mining and smelting

activities. Arsenic contamination in water depends on the level of human activity, the distance from the sources of pollution and the dispersion and fate of the arsenic that is released (USEPA, 2000). A recent estimation has placed the ratio of natural to anthropogenic atmospheric inputs of arsenic at 60:40 (Chilver et al., 1987).

Marine and freshwater organisms usually have much higher concentrations of arsenic in their structure than is present in the background of the surrounding water, but such “fish arsenic”(organic arsenic) is chemically and physiologically different from inorganic arsenate and arsenite. Arsenic occurs in seawater mainly as inorganic arsenate, at levels of approximately 2µg/L. In marine animals, levels of arsenic can be as high as 100 mg/kg (Shibata, 1992; Maher, 1988; Tamaki et al., 1992). The presence of organic arsenicals in marine organisms is commonly assumed to be due to the accumulation of compounds that have been synthesised from arsenate. This accumulation results in a much higher concentration of arsenic in the organism than that in the environmental water. Sometimes the level of this accumulation is astonishingly high. For example, in marine algae the mean arsenic concentration is 51.2±14.92 mg/kg, which is about 10000 times higher than that in the seawater. The fact that most of the arsenic in marine organisms is in organic forms indicates that arsenic is not simply entering the organism, but is transformed into organic forms within the organism.

Arsenic pollution in the aqueous environment mainly comes from arsenic containing waste-water produced from industrial processes. These wastes can pollute the soil and water sources. Serious pollution can cause acute arsenic poisoning incidents. In 1961, a mine in Guizhuo province in China produced arsenic-containing waste-water which contaminated the drinking water source and caused acute arsenic poisoning of residents (Zhao et al., 1963).

Acute toxicity

From human acute poisoning incidents, the LD₅₀ of arsenic has been estimated to range from 1 to 4 mg/kg (Vallee et al., 1960; Winship, 1984). This dose would correspond to a lethal dose range of 70 to 280 mg for 50% of adults weighing 70kg.

At non-lethal, but high acute doses, inorganic arsenic can cause gastroenterological effects, shock, neuritis (continuous pain) and vascular effects in humans (Buchanan, 1962).

Although certain arsenic compounds are toxic to humans, others are not. The toxicity of arsenic varies greatly with its chemical species, and its toxicity has a strong relationship with the status of arsenic in the component: inorganic arsenic is believed to be more toxic than organic arsenicals (Kaise et al., 1992). The LD50 values in rats for some arsenic species being (in mg/kg): arsine 3, arsenite 14, arsenate 20, monomethylarsonic acid (MMAA) 700-1800, dimethylarsinic acid (DMAA) 700-2600, arsenobetaine >10000 and arsenocholine 6500 (Mellan et al., 1956). The sequence of the toxicity potency of common arsenicals is:



Chronic toxicity

Long term exposure to arsenic results in chronic arsenic poisoning (arsenicosis). This has been reported in people who live in endemic areas with higher arsenic concentrations in drinking water or in burning coal. Arsenicosis has also been reported in people exposed to arsenic in the workplace. Skin lesions are the typical symptom of chronic arsenic poisoning. Among these changes the keratosis of the extremities of the limbs is very typical of arsenic poisoning. Melanosis and lesions are common changes. Chronic arsenic poisoning also causes damages including the respiratory system, digestive system, circulatory system, neural system and renal system. The most significant consequence is that inorganic arsenic causes cancers in various organs especially the skin, lung and bladder (ATSDR, 2000; IPCS, 2001). Episodes of chronic arsenic poisonings are reported around the world from America to Asia and Europe (Smedley et al., 2001).

A large number of adverse effects have been reported in humans after chronically exposure to drinking water highly contaminated with inorganic arsenic. The most

common symptoms of inorganic arsenic exposure appear on the skin and occur after 5-15 years of exposure equivalent to 700 µg/day for 70kg adult. They include alterations in pigmentation and the development of keratoses which are localized primarily on the palms of the hands, the soles of the feet and the torso. The presence of hyperpigmentation and keratoses on parts of the body not exposed to the sun is characteristic of arsenic exposure (Yeh, 1973; Tseng, 1977). Chronic exposure to inorganic arsenic is often associated with alterations in gastroenterological function (Morris et al., 1974; Nevens et al., 1990) and development of peripheral vascular disease (hardening of the arteries to the arms and legs, that can cause pain, numbness, tingling, infection, gangrene and clots). In Taiwan, black foot disease has been the most severe manifestation of this effect. Peripheral neuropathy may be present after long term exposure to inorganic arsenic in drinking water (Hindmarch et al., 1977; Southwick et al., 1983). People consuming arsenic contaminated water have an increased risk of diabetes (Lai et al., 1994; Rahman et al., 1998; Tseng et al., 2002). An increased risk of mortality from diabetes was reported in those occupationally exposed to arsenic in Sweden (Rahman and Axelson, 1995; Rahman et al., 1998).

Inorganic arsenic entering body by drinking water can cause multi-site cancer in human body. Asian, Mexican and South American populations with exposures to arsenic in drinking water generally at or above several hundred micrograms per liter are reported to have increased risks of skin, bladder, and lung cancer in Taiwan (Chen et al., 1985), in Argentina (Hopenhayn-Rich et al., 1996) and in Chile (Smith et al., 1998). The current evidence also suggests that the risks of liver and kidney cancer may also be increased following exposure to inorganic forms of arsenic. The first studies of dose-dependent effects of arsenic associated with skin cancer were observed in Taiwan (Tseng et al., 1968; 1977). Tseng's reports and other corroborating studies such as those by Albores et al. (1979) and Cebrian et al. (1983) on drinking water exposure and exposure to inorganic arsenic in medicines (Cuzick et al., 1982) and in pesticides (Roth, 1956) led the USEPA to use skin cancer as the endpoint to classifying inorganic arsenic as a human carcinogen (group A) by the oral route (USEPA, 1984). It has been estimated that more than 50 million people worldwide are at risk exposing to elevated levels of arsenic from the environment (Table 1).

Table 1. Episodes of arsenic caused poisonings and areas of potential arsenic contamination around the world

Country or Area	Population at risk	Sources of As exposure	Discover date	References
Bangladesh	50,000,000	Drinking water: artisan well	1980s	Saha (1984)
West Bengal, India	1,000,000	Drinking water: artisan well	1980s	Chowdhury et al., (1999)
Xinjiang, China	100,000	Drinking water: artisan well	1980s	Wang (1994); Cheng (1998)
Inner Mongolia, China	600,000	Drinking water: bores	1990s	Cheng (1998)
Guizhuo, China	20,000	Burning-coal	1950s	Cheng (1998)
Taiwan, China	200,000	Artisan well	1950s	Tseng and Chiou (1958)
Ronpibool, Thailand,	1,000	Water contaminated by tin mining waste	1980s	Choprapwon et al., (1995; 2001)
Mexica	400.000	Drinking water	1983	Cebrian et al., (1983)
Chile	437.000	Drinking water	1971	Sancha et al., (2001)
Argentina	2,000,000	Drinking water	1981	Sancha et al., (2001)
Bolivia	20,000	Drinking water	1997	Sancha et al., (2001)
Peru	250,000	Drinking water	1984	Sancha et al., (2001)
Vietnam	Millions	Drinking water	2001	Berg et al., (2001)
Western U S A	-	Drinking water	1988	Welch et al., (1988)
Hungary	220,000	Drinking water	1974	Sancha et al., (2001)
Romania	36.000	Drinking water (bores)	2001	Gurzau et al., (2001)

Recently Berg et al (2001) reported cases of high levels of arsenic contamination in an alluvial tract of the Red River in Vietnam as it passes through the city of Hanoi and the surrounding rural districts. The average arsenic concentration in the ground waters in this area was found to be 159µg/L, and varied from 1 to 3050 µg/L in rural ground

water samples from private small scale bores. In a highly affected rural area, the groundwater used directly for drinking water had an average concentration of 430 µg/L. The high arsenic concentrations found in the bores (48% above 50 µg/L and 20% above 150 µg/L) indicate that several million people consuming untreated groundwater might be at a considerable risk from chronic arsenic poisoning. [The WHO recommended drinking water guideline

Toxicity of organic arsenicals

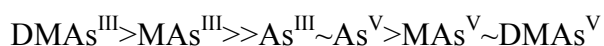
Arsenic exists in several forms that vary in toxicity and occurrence. It is important to consider those forms that can exert toxic effects and to which people may be exposed. Inorganic arsenic can have adverse effects; arsenite (As^{+3}) and arsenate (As^{+5}) are the most prevalent toxic forms found in drinking water. Volatile compounds, such as arsine (AsH_3) are very toxic; however arsine is not found in drinking water or food. Moreover, the primary organic forms (arsenobetaine and arsenocholine) found in fish and shellfish seem to have little or no toxicity.

Previous studies indicated that the organic arsenicals MMA and DMA that are the methylation metabolites of the inorganic arsenic were hundreds of times less likely to produce genetic changes in animal cells than inorganic arsenic. Organic arsenicals are less reactive in tissues, kill less cells and are more easily excreted in urine. But recent observations by researchers have challenged the idea that methylation transformation of inorganic arsenic is a detoxification mechanism (Petrick et al., 2000). These authors found that the organic arsenic - Monomethylarsonous acid (MMA^{III}) is more toxic than arsenite in Chang Human Hepatocytes. The mean LC_{50} based on LDH (leakage of lactate dehydrogenase) assay in phosphate media was 6µM for MMA^{III} and 68µM for arsenite. Using the assay for K^+ leakage in phosphate media, the mean LC_{50} was 6.3 µM for MMA^{III} and 19.8 µM for arsenite. The mean LC_{50} based on the XTT [the tetrazolium salt 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide inner salt] assay in phosphate media was 13.6µM for MMA^{III} and 164 µM for arsenite. The results of these three cytotoxicity assays reveal the following order of toxicity in Chang Human Hepatocytes:



These data demonstrate that MMA^{III} , an intermediate in inorganic arsenic methylation, is highly toxic and again raise the question as to whether methylation of inorganic arsenic is a detoxication process. MMA^{III} (Monomethylarsonous acid) has been detected for the first time in the urine of some humans exposed to inorganic arsenic in their drinking water (Aposhian et al., 2000). A speciation technique for eight arsenic compounds (special emphasis on identification and quantification of DMA^{III} and MMA^{III}) present in biological samples was developed (Le and Ma, 1997). The observations (Styblo et al., 1998; Petrick et al., 2000) that organic arsenic MMA^{III} is more toxic than inorganic arsenite indicate that (1) future studies concerning urinary arsenic profiles of arsenic-exposed humans must determine MMA^{III} concentrations, (2) previous studies of urinary profiles dealing with humans exposed to arsenic need to be re-examined and re-evaluated, and (3) since MMA^{III} is more toxic than inorganic arsenite, a re-examination is needed of the two hypotheses which hold that methylation is a detoxication process for inorganic arsenic and that inorganic arsenite is the major cause of the toxicity and carcinogenicity of inorganic arsenic.

Mass et al. (2001) reported that DMA^{III} is 386 fold more potent than As^{III} and summarised the genotoxicity potencies of methylated arsenicals *in vitro* (single cell gel assay) as follows:



The authors implied that trivalent methylated arsenic may be the toxic intermediate metabolites. However, the role of these methylated arsenicals in arsenic carcinogenicity *in vivo* remains unknown. The processes of metabolism of arsenicals *in vivo* are very complicated. There are several organs and systems involved in these processes.

Dimethylarsinic acid (DMA^V) - oxidative damage

Yamanaka et al. (1990) first presented a detailed metabolic pathway for arsenic in oxidative stress, as shown in Figure 1; dimethylarsine (a trivalent arsenic form) is a minor *in vivo* metabolite of DMA^V produced by a process of reduction *in vivo*. Dimethylarsine can react with molecular oxygen forming a (CH₃)₂As• radical and superoxide anion. This (CH₃)₂As• radical can add another molecule of molecular oxygen and form the (CH₃)₂AsOO• radical. A hydroxyl radical may be produced via cellular iron and other transition metals. Exposure to these free radicals can cause DNA damage such as DNA single-strand breaks (See Figure 1).

Matsui et al. (1999) reported the association of 8-OHdG with arsenic exposure in humans. They detected the role of oxidative DNA damage in human arsenic carcinogenesis by studying cases of arsenic-related skin neoplasms and arsenic keratosis as well as in 11 cases of arsenic unrelated Bowen's disease. The levels of 8-Hydroxy-2'-deoxyguanosine were significantly higher in arsenic-related skin neoplasms than in arsenic-unrelated Bowen's disease. 8-Hydroxy-2'-deoxyguanosine was also detected in normal tissue adjacent to arsenic-related Bowen's diseases lesions. Yamanaka et al. (2001) reported the urinary 8-OHdG levels (obtained by sampling from the urinary bladder) in mice gavaged with 720 mg/kg of DMA were increased to about 3, 3, and 8 times control levels at 3, 6, and 9h after treatment, respectively (Yamanaka et al., 2001). In a long-term rat carcinogenesis study, hepatic 8-OHdG levels were increased in DMA-treated rats, suggesting an elevated rate of free radical attack on DNA (Wanibuchi et al., 1997).

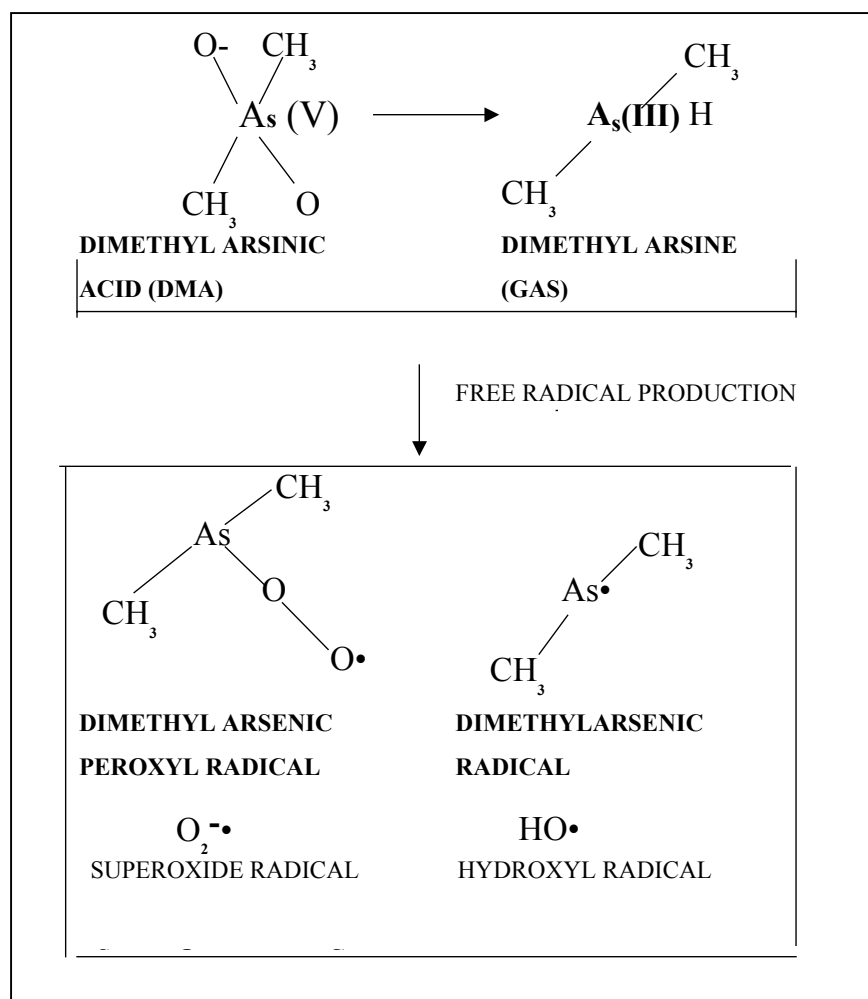


Figure 1. Free radicals from DMA^V. DMA^V may be reduced to a trivalent arsenic form, dimethylarsine. This minor pathway of arsenic metabolism may generate free radicals such as dimethylarsenic radical, dimethylarsenic peroxy radical, or indirectly generate superoxide and hydroxy radical (from Yamanaka et al., 1990).

Arsenic carcinogenicity

Evidence of inorganic arsenic being carcinogenic to humans is sufficient based on strong epidemiological studies (IARC, 1987; IPCS, 2001). However, the evidence to demonstrate arsenic is a complete carcinogen to animals is lacking until recently Ng (1998,1999) successfully demonstrated for the first time the formation of tumours in laboratory mice chronically exposed for over two years to inorganic arsenic in drinking water. This animal model is the most successful model for arsenic carcinogenesis effect and provides the possibility for further *in-vivo* study of arsenic carcinogenicity.

Cancer promoter

Much of the previous evidence has suggested that arsenic is a cancer promoter rather than a carcinogen in animal studies.

The urinary bladder has long been described as a major organ of chemical carcinogenesis, mostly because the excreted proximate metabolites of ingested chemicals being kept in the urinary bladder for relatively long duration with continuous exposure to the urothelium. DMA is the major excreted metabolite of ingested arsenic in numerous reports about human exposure to inorganic arsenic. Most of the absorbed DMA is excreted as unchanged DMA in the urine of various animal species. DMA itself acts as a urinary bladder carcinogen or particularly as a potent cancer promoter.

The indication that rat bladder was responsive to DMA-induced carcinogenesis first reported by Yamamoto et al. (1995; 1997). After a long time and many negative reports of arsenic carcinogenicity *in vivo* studies, their results are very significant, and showed that bladder cancer resulted from all four DMA-exposed F344/DuCrj rat groups after pre-exposed to cancer initiators. Multiple initiators were used through the carcinogenesis processes in several organs. In the initiated groups, DMA significantly enhanced the tumour induction in the urinary bladder, kidney, liver, and thyroid gland. Induction of preneoplastic lesions (glutathione S-transferase placental form-positive foci in the liver and atypical tubules in the kidneys) was also significantly increased in DMA-treated groups (see Table 2).

Urinary bladder tumours are so far the most frequently induced tumour type in the animal carcinogenesis studies. Following the work of Yamamoto et al (1995), at least 3 other research groups achieved the same result. For example, Wanibuchi et al. (1996) obtained the positive result of rat bladder tumours when only one initiator, N-butyl-N-(4-hydroxybutyl)nitrosamine was given followed by DMA exposure in 6-week-old male F344 rats. The development of preneoplastic lesions and tumours (papillomas and carcinomas) in the urinary bladder was enhanced by the treatment with DMA in a dose-dependent manner. A significant increase in multiplicity of

tumours (papillomas and carcinomas) was observed even at a relatively low concentration of DMA (10ppm). On the other hand, no preneoplastic lesions and tumours were observed in the rats treated with DMA alone. Wanibuchi and Fukushima (2000) also reported other results in relation to the structure-activity. In this work, rats were given control, arsenite (17.3ppm), MMA (187 ppm), DMA (184ppm), or TMAO (182ppm) respectively in their drinking water after initiated with N-butyl-N-(4-hydroxybutyl)nitrosamine developed 0.4, 0.3, 1.3, 3.8, and 0.9 bladder tumours per rat. The rat bladder tumour incidence was elevated at the $p < 0.05$ level for all three methylated arsenicals, with DMA clearly the most active chemical (See Table 2 and Table 3).

Recent studies have shown that tumours in various organs observed in humans in arsenic-endemic area can all be induced by arsenicals in animals as a cancer promoter. The kidney is the major route for the elimination of ingested arsenic from the human or animal body. Most of the metabolites are methylated, such as DMA, MMA and TMA. Most reports of the metabolism of ingested arsenic show accumulation of DMA in the kidney and liver. Studies also reported relatively high accumulation of ingested DMA in the lung and skin. Besides the results reported by Yamamoto et al (1995) and Wanibuchi et al (1996; 2000), several more studies report tumours involving many other organs. Yamanaka et al. (2001) used hairless mice receiving a topical application of dimethylbenz(a)anthracene (DMBA) with or without the exposure to ultraviolet B (UVB), a tumour promoter and the administration of DMA by the oral route promoted not only the formation of papillomas induced by DMBA alone but also the formation of malignant tumours induced by way of the formation of atypical keratoses by treatment with DMBA and UVB. In their previous report (Yamanaka et al., 2000), hairless mice (Hos: HR-1) were treated for 25 weeks with UVB light at 2KJ/m^2 twice per week with or without exposure to 400 or 1000 ppm of DMA in the drinking water. All three treated groups developed skin cancer, but the combination of UVB light and 1000 ppm of DMA in the drinking water induced more tumours per mouse during weeks 13 to 19 than did UVB light exposure alone. No statistically significant elevations in the percentage of tumour-bearing mice or tumours per mice occurred with the combination of UVB and 400 ppm DMA treatment versus UVB treatment alone.

In another study (Yamanaka et al., 1996), male ddY mice initiated with 4-nitroquinoline 1-oxide (4NQO) known as an initiator of lung tumours and subsequently exposed to 200 or 400 ppm DMA for 25 weeks in their drinking water developed lung tumours that were described as adenocarcinomas and adenosquamous carcinomas. The percentage of mice bearing tumour nodules was increased with the administration of both 4NQO and DMA than those mice given 4NQO alone, or given both 4NQO and glycerol. Furthermore, the average number of tumours nodules per mouse was significantly higher in mice administered DMA than those given glycerol (known to have potent promoting activity in lung tumourigenesis initiated by administration of 4NQO in ddY mice).

Wanibuchi et al. (1997) reported that in rats initiated with diethylnitrosamine DMA (25, 50, or 100 ppm) was active as a promoter in this experimental system for liver carcinogenesis based on the area stained for glutathione S-transferase placental form-positive foci. Rat hepatic ODC (Ornithine decarboxylase; its activity is often interpreted as a biomarker for cell proliferation) activity was increased after administration of both 10 and 50ppm DMA in the drinking water. In a recent structure-activity study (Wanibuchi and Fukushima, 2000), rats initiated with diethylnitrosamine developed about 1.5, 2.5, 2.4, and 2.5 glutathione S-transferase placental form-positive foci/cm² following exposure to promotional regimens of control, MMA, DMA, or TMAO, respectively. The number of the liver foci per area was statistically elevated at the $p < 0.05$ for all three methylated arsenicals (Table 2).

Transgenic animals have been used for developing arsenic carcinogenesis model. Since the mechanisms of arsenic carcinogenesis are not clear, different transgenic animals have been tested in developing the manifestation of the arsenic carcinogenic effects. Genetically altered rodents were used in anticipation for the rapid identification of carcinogens. The use of these models holds great promise for shortening the length of the bioassay and reducing its cost. The enhancement of the sensitivity of the models could allow agents to be tested at lower doses more relevant to human exposure.

The p53 gene is a tumour suppression gene, it is a very important gene in preventing tumour occurrence. Salim et al. (1999) reported the use of p53^{+/-} mice, to which DMA was given to p53 heterozygous knockout and C57BL/6T wild-type male mice

in their drinking water for 80 weeks. Exposure to 50 or 200 ppm of DMA caused an increase in the total numbers of spontaneous tumours in wild-type mice and earlier induction of tumours in the p53 knockout mice.

Ornithine decarboxylase (ODC), an important enzyme in the polyamine biosynthetic pathway, is aberrantly regulated in epithelial tumours of rodents and humans. In murine skin, it has been shown that ODC overexpression provides a sufficient condition for tumour promotion. K6/ODC transgenic mice in which ODC overexpression was targeted to hair follicle keratinocytes might provide a sensitive model for identifying genotoxic carcinogens (Chen et al., 2000). A two-stage mouse skin carcinogenesis experiment in K6/ODC mice was performed by using the initiator 7,12-dimethylbenz[a]anthracene (DMBA, 50 µg, single application), 12-O-tetradecanoylphorbol-13-acetate (TPA, 5 µg twice per week) and DMA (3.6 mg twice per week). Twenty weeks after initiation, the average number of tumours per mouse was 20.7, 19.4, 9.7, and 0.0 in the four treatment groups of DMBA → TPA, DMBA → DMA, DMBA → control cream, and acetone vehicle → DMA, respectively. The DMBA → TPA treatment group responded with an increased tumour incidence about 2 weeks faster than the DMBA → DMA group. Microscopically, most tumours in the three responding treatment groups were squamous papillomas, and some squamous carcinomas with disorderly arrangement and atypical nuclei (Morikawa et al., 2000).

Table 2. Organic Arsenicals in Various Animal Carcinogenicity Models

Arsenical Type	Animal Species & Strains	Dose & Treatment/Regime	Exposure Duration	Exposure Pathway	Results	Role of As	Reference
DMA	Hos:HR-1 female hairless mice	400 & 1000ppm DMA+UVB	25 weeks	Drinking water	Skin Cancer	Promotor	Yamanaka et al., 2000
DMA	Hos:HR-1 female hairless mice	DMA+ DMBA±UVB	25 weeks	topical DMA drinking water	Skin tumour	Promotor	Yamanaka et al., 2001
DMA	F344/DuCrj male rats	50,100,200,400ppm +DEN,MNU,DMH BBN,DHPN	30 weeks	Drinking Water & i.p(Initiator)	Bladder,Kidney Liver &Thyroid Tumour	Promotor	Yamamoto et al., 1995
DMA	F344 male rats	DMA(0, 2, 10, 25, 50 and 100 ppm)±BBN	32 weeks	Dinking water	Bladder tumour	Promotor	Wanibuchi et al., 1996
DMA, MMA,TMAO	F344 male rats	MMA(187ppm) DMA(184) TMAO (182ppm)+BBN		Drinking water	Bladder tumour	Promotor	Wanibuchi et al.,2000
DMA	ddY male mice	200 or 400 ppm DMA+4NQO	25 weeks	Drinking water	Lung tumour	Promotor	Yamanaka et al., 1996
DMA	F344 male rats	25, 50, or 100ppm DMA +DEN	8 weeks	Food and Drinking water . Den by i.p	Positive in Ito test	Promotor	Wanibuchi et al., 1997
MMA, TMAO	F344male rats	MMA,TMAO + DEN			Positive in Ito test	Promotor	Wanibuchi et al., 2000
DMA	P53 ⁺ &C57BL/6T male mice	50 or 200 ppm	80 weeks	Drinking water	Tumour increase & early induction of tumour	Promotor	Salim et al., 1999
DMA	K6/ODC male mice	DMA 3.6 mg twice/week +DMBA or TPA	20 weeks	Topical application	Skin tumour accelerated	Promotor	Morikawa et al., 2000
DMA	A/J male mice	50, 200 or 400 ppm	25-50 weeks	Drinking water	Lung tumours	Carcinogen	Hayashi et al.,1998
DMA	F344/DuCrj male rats	0, 12.5, 50, 200 ppm (negative in 0 & 12.5ppm)	104 weeks	Drinking water	Bladder tumour	Carcinogen	Wei et al.,1999
DMA	F344 female rats	100ppm	24 months	Food	Bladder tumours	Carcinogen	Kitchin 2001
DMA	B6C3F1 female mice	500ppm	24 months	Drinking water	Fibrosarcomas in multiple organs	Carcinogen	Kitchin 2001
DMA	K6/ODC male mice	10, 100ppm DMA	5 months	Drinking water	Skin tumour	Carcinogen	Chen et al.,2000

DMA=Dimethylarsinic acid; MMA=Monomethylarsonic acide; TMAO=Trimethylarsine oxide; As(III)=Arsenite; As(V)= Arsenate; BBN= N-butyl-N-(4-hydroxybutyl)nitrosamine; DEN=diethylnitrosamine; MNU=N-methyl-N-nitrosourea; DMH=1,2-dimethylhydrazine; DHPN= N-bis(2-hydroxypropyl)nitrosamine; 4NQO=4-nitroquinoline-1-oxide; DMBA=7,12-dimethylbenz[a]anthracene; TPA=12 -O-tetradecanoylphorbol-13-acetate; ODC=ornithine decarboxylase; UVB= Ultraviolet B

Complete carcinogen

Hayashi et al. (1998) used A/J mice given 50, 200, or 400 ppm DMA in drinking water for either 25 or 50 weeks. At 25 weeks there were no DMA-related pulmonary tumours. Alveolar adenomas were not increased by DMA administration at 50 weeks. However, mice fed with the highest dosage (400 ppm) of DMAA for 50 weeks produced more pulmonary tumours than untreated mice (mean number per animal 1.36 versus 0.50; $p < 0.05$). Increased numbers of lung hyperplasia, papillary adenomas, and adenocarcinomas were observed after treatment with 50, 200, or 400

ppm of DMA for 50 weeks. In this experiment, DMA is acting as a complete carcinogen in A/J mice which known to be susceptible to developing pulmonary tumours that are normally seen in humans exposed to arsenic.

Wei et al. (1998) also tested DMA as a complete carcinogen and found it to be carcinogenic to rat urinary bladder at 50 and 200 ppm. Total of 144 F344 rats (10 weeks old at the start) were used to determine the carcinogenicity of dimethylarsinic acid (DMA). DMA (purity 100%) concentrations of 200, 50, 12.5 and 0 ppm were given to 4 groups of rats in the drinking water respectively for 104 weeks. From weeks 97 to 104, urinary bladder tumours were observed in 12 of 31, 8 of 31 and none of 33 in groups 1-3, respectively. No bladder tumours were observed in the negative control group 4.

An unpublished study work (cited by Kitchin, 2001) was performed by Life Sciences Research of Israel in support of the U.S. EPA pesticide registration for cacodylic acid (USEPA, 1994). In this study, female F344 rats exposed for 2 years to up to 100 ppm DMA in their food developed dose-related transitional cell bladder tumours with bladder hyperplasia observed in both sexes. Control female rats had 0/59 incidence of bladder papillomas and carcinomas, while female rats exposed to 100 ppm DMA had 10/58 incidence (17%, $p < 0.01$). Of the female rats that responded to DMA exposure with bladder tumours, six were classified as carcinomas and four were classified as papillomas. Female B6C3F1 mice exposed to up to 500ppm DMA in their drinking water showed only an increase in fibrosarcomas in multiple organs.

ODC (ornithine decarboxylase) activity is induced in epithelial tumours in both rodents and humans. ODC has been extensively studied in mouse skin and rat liver carcinogenesis systems and ODC induction is considered as a biomarker for cancer promotion. Chen et al. (2000) tested ten known carcinogens or noncarcinogens using the K6/ODC transgenic mouse model. In K6/ODC mice given either 10 ppm DMA, 100 ppm DMA, or 10 ppm arsenite in their drinking water for 5 months, 8, 22, or 15% of the mice developed skin squamous papillomas, respectively. No tumours were observed in the control group of K6/ODC transgenic mice not exposed to arsenic and in nontransgenic littermates given 100 ppm DMA.

Table 3. Inorganic arsenicals in various animal carcinogenicity models

Arsenical Type	Animal Species & Strains	Dose & Treatment/ Regime	Exposure Duration	Exposure Pathway	Results	Role of As	Reference
As(III)	Rats	250ppm	24 months	Diet	Negative	-	Byron et al 1967
As(V)	Rats	400ppm	24 months	Diet	Negative	-	Byron et al 1967
As(III)	Rats	416ppm	29 months	Diet	Negative	-	Kroes et al 1974
As(III)/ As(V)	Beagles	5 - 125ppm	24 months	Diet	Negative	-	Byron et al 1967
As(V)	Cynomologus Monkeys	0.1mg/kg/day 5 days/week	15 years	Diet	Negative	-	Thorgeirsson et al 1994
As(III)	Rats	5 mg/L	lifetime	Drinking water	Negative	-	Schroeder et al 1968
As(V)	Rats	5 mg/L	lifetime	Drinking water	Negative	-	Kanisawa et al 1969
As(III)	Inbred C ₃ H Mice	10 mg/ L	16 weeks	Drinking water	Lower tumour rate than controls	-	Schrauzer et al 1974
As(III)	F344 rats	As(III)(17.3ppm)		Drinking water	Bladder tumour	Promotor	Wanibuchi et al., 2000
As(III)	Tg.AC female mice	200 ppm As(III) + TPA	22 weeks As ⁺³ pretreated 4 weeks	Drinking water	Skin tumour increased	Promotor	Germolec et al., 1998
As ₂ O ₃	mice	1.2mgAs/kg (mother) 5 ug As/pup		subcutaneous	Lung adenoma in off-spring mice	Carcinogen	Rudnay et al., 1981
As ₂ O ₃	Hamsters	3mg As/kg Weekly	15 weeks	Intratracheal instillation	Respiratory tract tumours	Carcinogen	Pershagen et al., 1984
As(III)	Rats	8 mg/capsule		Surgical implantation in stomach	Stomach adenocarcinomas	Carcinogen	Katsnelson et al., 1986
As(III)	Rats	160 ppm As/L+ i.p 30mg/kg of DEN	175 days	Drinking water	Renal tumours	Carcinogen	Shirachi et al., 1983
As(III)	p53 ^{+/-} male mice	50 ppm +Choline- Deficient Diet + expose to p-cresidine	26 weeks	Diet	Increase bladder tumour	Cocarcinogen	Popovicova et al., 2000
As(V)	C57BL/6J and MT- female mice	500ug/L	up to 26 months	Drinking water	Multiple tumours	Carcinogen	NG et al., 1999
As(III)	K6/ODC male mice	10ppm As(III)	5 months	Drinking water	Skin tumour	Carcinogen	Chen et al., 2000
As(III)	Hairless Skh1 mice	10mg/L As(III)+1.7KJ/m ² UVR 3 times weekly	26 weeks	Drinking water	Skin tumour	Cocarcinogen	Rossmann., 2001

As(III)= Arsenite; As(V)= Arsenate; DEN=diethylnitrosamine; TPA=12-O-tetradecanoylphorbol-13-acetate; ODC=ornithine decarboxylase; UVB= Ultraviolet B; UVR=Ultraviolet radiation; MT=Metallothionien knock-out.

Mode of action for arsenic carcinogenicity

The mode of action for arsenic carcinogenicity (DMA included) is still unclear. Although earlier studies have failed to come up with a successful arsenic tumour model, the effort for the development of arsenic tumours of animal models has provided a lot of information on arsenic genotoxic effects. An *in-vitro* study has shown that arsenic induces chromosome aberrations and DNA-protein cross-links (IARC, 1980). Reactive oxygen species (ROS) produced by dimethylarsenic acid, one

of the major metabolites of inorganic arsenics, were suggested to be involved in the generation of DNA damage (Yamanaka et al., 1989). Yamanaka and Okada (1994) also assumed that of the two radicals, dimethylarsenic peroxy radical and the superoxide anion radical produced from the reaction between molecular oxygen and dimethylarsine, the dimethylarsine peroxy radical plays a dominant role(s) in causing DNA damage, and at least causing DNA single-strand breaks. Dimethylarsenic acid has the potential to promote rat liver carcinogenesis, possibly via a mechanism involving stimulation of cell proliferation and DNA damage caused by ROS (Wanibuchi et al., 1997). Sodium arsenite produced DNA-protein crosslinks and arsenic mediated DNA-protein interactions may play a major role in arsenic carcinogenesis (Dong and Luo, 1993). Arsenic was suggested to enhance the development of skin neoplasias via chronic stimulation of keratinocyte-derived growth factors and may be a rare example of a chemical carcinogen that acts as a co-promoter (Germolec et al., 1998).

Unlike some heavy metals such as cadmium and nickel that bind directly to the DNA, arsenic showed no direct interaction with DNA. The mechanism of the action of arsenic as a carcinogen is not well understood but the affecting process can be inferred from data that suggest arsenic causes large deletion (Hei et al., 1998) or methylation changes of DNA (Mass and Wang, 1997; Zhao et al., 1997) that could affect gene expression. The inhibition of the DNA repair function has also been suggested as one of the mechanisms of inorganic arsenic carcinogenicity (Hartwig, 1998; Tran et al., 2002). The oxidative DNA damage in skin neoplasms related to arsenic attack is suggested by the *in-vivo* evidence in arsenic-induced Bowen's disease and the cases of bladder tumours among Taiwanese who lived in areas of high arsenic-contaminated artisan well drinking water (Matsui et al., 1999).

In conclusion, sufficient evidence to establish that both inorganic arsenic and its methylated forms (DMA and MMA) are either complete carcinogens or cancer promoters or both although the mode of action for their carcinogenic effects require further research. The synergistic effects of DMA / MMA and others carcinogenic

Watervolatility of PCDD/Fs

compounds have been reported extensively. The potential synergistic effect between DMA and dioxins remains unknown.

Appendix II: **References**

- Albores, A., Cebrian, M.E., Tellez, I., Valdez, B., 1979. Comparative Study of Chronic Hydroarsenicism in Two Rural Communities in the Region Lagunra of Mexico. (in Spanish). *Bol. Oficina Sanit. Panam.* 86, 196-205.
- Aposhian, H.V., Gurzau, E.S., Le, X.C., Gurzau, A., Healy, S.M., Lu, X.F., Ma, M.S., Yip, L., Zakharyan, R.A., Maiorino, R.M., Dart, R.C., Tircus, M. G., Gonzalez-Ramirez, D., Morgan, D.L., Avram, D., Aposhian, M.M., 2000. Occurrence of Monomethylarsonous Acid in Urine of Humans Exposed to Inorganic Arsenic. *Chem. Res. Toxicol.* 13, 693-697.
- ATSDR (Agency for Toxic Substances and Disease Registry) 2000. Toxicological Profile for Arsenic. U.S. Department of Health and Human Services, Agency for Toxic Substances and Disease Registry, Atlanta, GA.
- Berg, M., Tran, H.C., Nguyen, T.C., Pham, H.V., Schertenleib, R., Giger, W., 2001. Arsenic Contamination of Groundwater and Drinking Water in Vietnam: A Human Health Threat. *Environmental Science and Technology.* 35, 2621-2626.
- Buchanan, W. D., 1962. *Toxicity of Arsenic Compounds.* Elsevier Scientific Publishers. pp v-viii.
- Byron, W.R., Bierbower, G.W., Brouwer, J.B., Hansen, W.H., 1967. Pathologic Changes in Rats and Dogs from Two-year Feeding of Sodium Arsenite or Sodium Arsenate. *Toxicol. Appl. Pharmacol.* 10, 132-147.
- Cebrian, M. E., Albores, A., Aguilar, M., Blakely, E., 1983. Chronic Arsenic Poisoning in the North of Mexico. *Human Toxicology.* 2, 121- 133.
- Chen, C.J., Chuang, Y.C., Lin, T.M., Wu, H.Y., 1985. Malignant Neoplasms Among Residents of A Blackfoot Disease-Endemic Area in Tainwan: High-arsenic Artesian Well Water and Cancers. *Cancer. Res.* 45, 5895-5899.
- Chen, Y., Lousis, C.M., Susan, K.G., Sawicki, J.A., O'Brien, T.G., 2000. K6/ODC Transgenic Mice as a Sensitive Model for Carcinogen Identification. *Toxicology Letters.* 116, 26-35.
- Cheng, Z., Zhang, L.L., Yang, F.S., Lu, Z.M., Zhang, X.L., 1998. The Epidemiology and Control of Arsenicosis in China. *Journal of Chinese Endemic Disease Control.* (in Chinese) 13, 342-345.
- Chilvers, D. C., Peterson, P. J., 1987. Global cycling of arsenic. In: *Hutchinson TC & Meema KM eds. Lead, Mercury, Cadmium and Arsenic in the Environment.* Chichester, John Wiley & Sons, pp. 279-303. (SCOPE 31).
- Choprapwon, C., 1995. Chronic Arsenic Poisoning in Ronpibool District, Nakorn Srithammarat Province, Southern Thailand: How Should We Balance Between Economics and Health. Second International Conference on Arsenic Exposure and Health Effects.
- Choprapwon, C., Porapaktham, Y., 2001. Occurrence of Cancer in Arsenic Contaminated Area, Ronpibool District, Nakorn Srithmmarat Province, Thailand. *Arsenic Exposure and Health Effects IV.* 201-206.
- Chowdhury, U.K., 1999. The Condition of Arsenic Contamination of Underground Water and Prevalent of Arsenicosis in Residents in Bangladesh and West Bangel. Abstract Book of Pan-Asia Pacific Conference on Fluoride and Arsenic Research. Shengyang, China. August, 16-20.
- Clifford, D., Zhang, Z., 1994. Arsenic Chemistry and Speciation. *American Water Works Association Annual Conference.* New York, NY. June 19-23.

- Cullen, W.R., Reimer, K.J., 1989. Arsenic Speciation in the Environment. *Chem.Rev.* 89, 713-764.
- Cuzick, J., Evans, S., Gillman, M., Price-Evans, D.A., 1982. Medicinal Arsenic and Internal Malignancies. *British Journal of Cancer.* 45, 904-911.
- Dong, J.T., Luo, X.M., 1993. Arsenic-induced DNA-strand Breaks Associated with DNA-protein Crosslinks in Human Fetal Lung Fibroblasts. *Mutat Res.* 302, 97-102.
- Germolec, D.R., Spalding, J., Hsin-Su, Yu., Chen, G.S., Simeonova, P.P., Humble, M.C., Bruccoleri, A., Boorman, G.A., Foley, J.F., Yoshida, T., Luster, M.I., 1998. Arsenic Enhancement of Skin Neoplasia by Chronic Stimulation of Growth Factors. *American Journal of Pathology.* 153, 1775-1785.
- Gurzau, E.S., Gurzau, A.E., 2001. Arsenic in Drinking Water from Groundwater in Transylvania, Romania: an Overview. *Arsenic Exposure and Health Effects IV.* 181-184.
- Hartwig, A., 1998. Carcinogenicity of Metal Compounds: Possible Role of DNA Repair Inhibition. *Toxicol Lett.* 102-103, 235-239.
- Hayashi, H., Kanisawa, M., Yamanaka, K., Ito, T., Udaka, N., Ohji, H., Okudela, K., Okada, S., Kitamura, H., 1998. Dimethylarsinic Acid, A Main Metabolite of Inorganic Arsenics, Has Tumourigenicity and Progression Effects in the Pulmonary Tumours of A/J Mice. *Cancer Letters.* 125, 83-88.
- Hindmarsh, J. T., McLetchie, O.R., Heffernan, L.P.M., Hayne, O.A., Ellenberger, H.A., McCurdy, R.F., Thiebaut, H.J., 1977. Electromyographic Abnormalities in Chronic Environmental Arsenicalism. *Analytical Toxicology.* 1, 270-276.
- Hopenhayn-Rich, C., Biggs, M.L., Fuchs, A., Bergoglio, R., Tello, E.E., Nicolli, H., Smith, A.H., 1996. Bladder Cancer Mortality Associated With Arsenic in Drinking Water in Argentina. *Epidemiology.* 7, 117-124.
- IARC (International Agency for Research on Cancer), 1980. Some Metals and Metallic Compounds. *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans.* Vol.23. Lyon, France, International Agency for Research on Cancer.
- IARC (International Agency for Research on Cancer), 1987. Arsenic and Arsenic Compounds. pp. 100-206 in IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Overall Evaluations of Carcinogenicity: An Updating of IARC Monographs 1-42, Suppl.7. Lyon, France, International Agency for Research On Cancer.
- IPCS (International Programme on Chemical Safety), 2001. Environmental Health Criteria 224. Arsenic and Arsenic Compounds (Second Edition). *World Health Organization.* Geneva.
- Irgolic, K. J., 1994. Determination of Total Arsenic and Arsenic Compounds in Drinking Water. In *Arsenic: Exposure and Health.* Chappell W R, Abernathy C O & Cothorn, C R, Eds. Northwood, U.K., *Science and Technology Letters:* pp.51-60.
- Kaise, T., Fukui, S., 1992. The Chemical Form and Acute Toxicity of Arsenic Compounds in Marine Organisms. *Appl Organomet Chem.* 6, 155-60.
- Kanisawa, M., Schroeder, H.A., 1969. Life Term Studies on the Effect of Trace Elements on Spontaneous Tumours in Mice and Rats. *Cancer. Res.* 29, 892-895.
- Katsnelson, B.A., Neizvestnova, Y.M., Blokhin, V.A., 1986. Stomach Carcinogenesis Induction by Chronic Treatment with Arsenic (Russ.). *Vopr. Onkol.* 32, 68-73.
- Kitchin, K.T., 2001. Recent Advances in Arsenic Carcinogenesis: Modes of Action, Animal Model Systems, and Methylated Arsenic Metabolites. *Toxicology and Applied Pharmacology.* 172, 249-261.

- Kroes, R., Van, Logten, M.J., Berkvens, J.M., De Vries, T., van Esch, G.J., 1974. Study On the Carcinogenicity of Lead Arsenate and Sodium Arsenate and on the Possible Synergistic Effect of Diethylnitrosamine. *Food. Cosmer. Toxicol.* 12, 671-679.
- Lai, M. S., Hsueh, Y.M., Chien, C.J., Shyu, M.P., Chen, S.Y., Kuo, T.L., Wu, M.M., Tai, T.Y., 1994. Ingested Inorganic Arsenic and Prevalence of Diabetes Mellitus. *American Journal of Epidemiology.* 139, 484-492.
- Le, X.C., Ma, M.S., 1997. Speciation of Arsenic Compounds by using Ion-pair Chromatography with Atomic Spectrometry and Mass Spectrometry Detection. *Journal of Chromatography A.* 764, 55-64.
- Maher, W., Butler, E., 1988. *Appl. Organomet. Chem.* 2,191-214.
- Mass, M.J., Tennant, A., Roop, B.C., Cullen, W.R., Styblo, M., Thomas, D.J., Kligerman, A.D., 2001. Methylated Trivalent Arsenic Species are Genotoxic. *Chem. Res. Toxicol.* 14, 355-361.
- Mass, M.J., Wang, L., 1997. Arsenic Alters Cytosine Methylation Patterns of the Promoter of the Tumour Suppressor Gene *p53* in Human Lung Cells: a Model for a Mechanism of Carcinogenesis. *Mutat. Res.* 386, 263-277.
- Matsui, M., Nishigori, C., Toyokuni, S., Takasa, J., Akaboshi, M., Ishikawa, M., Imamura, S., Miyachi, Y., 1999. The Role of Oxidative DNA Damage in Human Arsenic Carcinogenesis: Detection of 8-Hydroxy-2'-Deoxyguanosine in Arsenic-Related Bowen's Disease. *The Journal of Investigative Dermatology.* 113, 26-31.
- Morikawa, T., Wanibuchi, H., Morimura, K., Ogawa, M., Fukushima, S., 2000. Promotion of Skin Carcinogenesis by Dimethylarsinic Acid in Keratin (K6)/ODC Transgenic Mice. *Jpn. J. Cancer. Res.* 91, 579-581.
- Morris, J. S., Schmid, M., Newman, S., Scheuer, P.J., Sherlock, S., 1974. Arsenic and Noncirrhotic Portal Hypertension. *Gastroenterology.* 66, 86-94.
- Nevens, F., Fevery, J., Steenbergen, W.V., Sciote, R., Desmet, V., Groote, J.De., 1990. Arsenic and Noncirrhotic Portal Hypertension: A Report of Eight Cases. *Hepatology.* 11, 80-85.
- Ng, J.C., 1999. Speciation, Bioavailability and Toxicology of Arsenic in the Environment. Ph.D. Thesis, University of Queensland, Brisbane, QLD, Australia.
- Ng, J.C., Qi, L., Wang, J.P., Xiao, X.L., Shahin, M., Moore, M.R., Prakash, A.S., 2001. Mutations in C57Bl/6J and Metallothionein Knock-out Mice induced by Chronic Exposure of Sodium Arsenate in Drinking Water. *Arsenic Exposure and Health Effects IV*, 231-242.
- Ng, J.C., Seawright, A.A., Qi, L., Garnett, C.M., Moore, M.M., Chriswell, B., 1998. Tumours in Mice Induced by Chronic Exposure of High Arsenic Concentration in Drinking Water [abstract]. Pp. 28: In *Book of Abstracts of the Third International Conference on Arsenic Exposure and Health Effects*, July 12-15, San Diego, Calif.
- Pershagen, G., Nordberg, G., Bjorklund, N.E., 1984. Carcinomas of the Respiratory Tract in Hamsters Given Arsenic Trioxide and / or Benzo(a)pyrene by the Pulmonary Route. *Environ. Res.* 34, 227-241.
- Petrick, J. S., Ayala-Fierro, F., Cullen, W. R., Carter, D. E., Aposhian, H. V., 2000. Monomethylarsonous acid (MMAIII) is more toxic than arsenite in Chang human hepatocytes. *Toxicology and applied pharmacology*, 163, 203-207.

- Petrick, J.S., Jagadish, B., Mash, E.A., Aposhian, H.V., 2001. Monomethylarsonous Acid (MMA^{III}) and Arsenite: LD 50 in Hamsters and *in vitro* Inhibition of Pyruvate Dehydrogenase. *Chem Res Toxicol.* 14, 651-656.
- Popovicova, J., Moser, G.J., Goldsworthy, T.L., Tice, R.R., 2000. Carcinogenicity and Co-carcinogenicity of Sodium Arsenite in *p53*^{+/-} Male Mice. *Toxicologist.* 54, 134.
- Rahman, M., Axelson, J.O., 1995. Diabetes Mellitus and Arsenic Exposure: a Second Look at Case-Control Data from a Swedish Copper Smelter. *Occupational Environmental Medicine.* 52, 773-774.
- Rahman, M., Tondel, M., Ahmad, S. A., Axels, C., 1998. Diabetese Mellitus Associated with Arsenic Exposure in Bangladesh. *American Journal of Epidemiology.* 148, 198-203.
- Rossmann, T.G., Uddin, A.N., Burns, F.J., Bosland, M.C., 2001. Arsenite is a Cocarcinogen with Solar Ultraviolet Radiation for Mouse Skin: An Animal Model for Arsenic Carcinogenesis. *Toxicol. Appl. Pharmacol.* 176, 64-71.
- Roth, F., 1956. Concerning Chronic Arsenic Poisoning of the Moselle Wine Growers with Special Emphasis on Arsenic Carcinomas. *Krebsforschung.* 61, 287-319.
- Rudnay, P., Borzsonyi, M., 1981. The Tumourigenic Effect of Treatment with Arsenic Trioxide (Hung). *Magyar. Onkol.* 25, 73-77.
- Saha, K.C., 1984. Melanokeratosis from Arsenic Contaminated Tubewell Water. *Indian J Dermatol.* 29, 37-46.
- Salim, E.I., Wanibuchi, H., Yamamoto, S., Morimura, K., Fukushima, S., 1999. Carcinogenicity of Dimethylarsinic Acid (DMAA) in *p53* Knockout and Wild Type C57BL/6T Mice. *Proc.Am.Assoc. Cancer.Res.* 40, 2335.
- Sancha, A.M., Castro, M.L., 2001. Arsenic in Latin America: Occurrence, Exposure, Health Effects and Remediation. *Arsenic Exposure and Health Effects IV.* 87-96.
- Schrauzer, G.N., Ishmael, D., 1974. Effects of Selenium and Arsenic on the Genesis of Spontaneous Mammary Tumours in Inbred C₃H Mice. *Ann. Clin. Lab. Sci.* 4, 441-447.
- Schroeder, H.A., Kanisawa, M., Frost, D.V., Mitchener, M., 1968. Germanium, Tin and Arsenic in Rats: Effect on Growth, Survival, Pathological Lesions and Life Span. *J. Nutrition.* 96, 37-45.
- Shibata, Y., Morita, M., Fuwa, K., 1992. Selenium and Arsenic in Biology. *Adv.Biophys.* 28, 31-80.
- Shirachi, D.Y., Johansen, M.G., McGowan, J. P., Tu, S. H., 1983. Tumourigenic Effect of Sodium Arsenite in Rat Kidney. *Proc. West. Pharmacol. Soc.* 26, 413-415.
- Smedley, P.L., Kinniburgh, D.G., Huq, I., Luo, Z.D., Nicolli, H.B., 2001. International Perspective on Naturally Occurring Arsenic Problems in Groundwater. *Arsenic Exposure and Health Effects IV.* 9-25.
- Smith, A. H., Goycolea, M., Haque, R., Biggs, M.L., 1998. Marked Increase in Bladder and Lung Cancer Mortality in a Region of Northern Chile Due to Arsenic in Drinking Water. *American Journal of Epidemiology.* 127, 660-669.
- Southwick, J. W., Western, A. E., Beck, M.M., Whitley, T., Isaacs, R., Petajan, J., Hansen, C. D., 1983. An Epidemiological Study of Arsenic in Drinking Water in Millard County, Utah. In *Arsenic: Industrial, Biomedical, Environmental Perspectives.* Lederer, W. H and Fensterheim, R. J., Eds. New York, Van Nostrand Reinhold: pp.210-225.
- Styblo, M., Vega, L., Germolec, D. R., Luster, M. I., Del-Razo, L. M., Wang, C., Cullen, W. R., Thomas, D.J., 1998. Metabolism and toxicity of arsenicals in

- cultured cells. In *Arsenic Exposure and Health Effects: Proceedings of the Third International Conference on Arsenic Exposure and Health Effects* (Chappell W R, Abernathy C O, and Calderon R L, Eds.) San Diego, CA, Elsevier Science Ltd., Oxford U K. pp311-323.
- Tamaki, S., Frankenberger, W. T., 1992. Environmental Biochemistry of Arsenic. *Jr. Rev. Environ. Contam. Toxicol.* 124, 79-110.
- Thorgeirsson, U.P., Dalgard, D.W., Reeves, J., Adamson, R.H., 1994. Tumour Incidence in a Chemical Carcinogenesis Study of Nonhuman Primates. *Regul. Toxicol. Pharmacol.* 19,130-151.
- Tran, H.P. Prakash A.S., Barnard, R., Chiswell, B., Ng, J.C., 2002. Arsenic inhibits the repair of DNA induced by benzo(a)pyrene. *Toxicology Letters*, 133, 59-67.
- Tseng, C.H., Tseng, C.P., Chiou, H.Y., Hsueh, Y.M., Chong, C.K., Chen, C.J., 2002. Epidemiologic Evidence of Diabetogenic Effect of Arsenic. *Toxicology Letters*. 133, 69-76.
- Tseng, W. P., 1977. Effects and Dose-Response Relationships of Skin Cancer and Blackfoot Disease with Arsenic. *Environmental Health Perspectives*. 19, 109-119.
- Tseng, W. P., Chu, H.M., How, S.W., Fong, J.M., Lin, C.S., Yeh, S., 1968. Prevalence of Skin Cancer in an Endemic Area of Chronic Arsenicism in Taiwan. *Journal of the National Cancer Institute*. 40, 453-463.
- USEPA, 1984. Health Assessment Document for Inorganic Arsenic. Final Report. EPA 600/8-83/021F. U.S. Environmental Protection Agency. Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, Ohio.
- USEPA, 1994. *Carcinogenicity Peer Review of Cacodylic Acid*. Memorandum from S. Malish and E. Rinde to C. Giles-Parker and J. Ellenberger. U.S. EPA, Office of Pesticides and Toxic substances, Washington, D.C.
- USEPA, 2000 Federal Register. 65(121) /Thursday, June 22, 2000.
- Vallee, B.L., Ulmer, D.D., Wacker, W.E.C., 1960. Arsenic Toxicology and Biochemistry. *AMA Arch. Ind.Med.* 21, 56-75.
- Wang, L. F., Huang, J. Z., 1994. Chronic Arsenism From Drinking Water in Some Areas of Xinjiang, China. In: *Arsenic in the Environment, Part II: Human Health and Ecosystem Effects*. Edited by J O Nriagu, John Wiley & Sons, Inc, 159-172.
- Wang, L.F., 1997. The Endemic Arsenic Poisoning and Blackfoot Disease. People's Health Publishing House of Xinjiang, Urumqi, P. R. China (in Chinese).
- Wanibuchi, H., Fukushima, S., 2000. Carcinogenicity of an Organic Arsenic, Dimethylarsenic Acid in Animals. *XXIII International Congress of the International Academy of Pathology and 14 th World Congress of Academic and Environmental Pathology*, Nagoya, Japan.
- Wanibuchi, H., Hori, T., Meenakshi, V., Ichihara, T., Yamamoto, S., Yano, Y., Otani, S., Nakae, D., Konishi, Y., Fukushima, S., 1997. Promotion of Rat Hepatocarcinogenesis by Dimethylarsinic Acid: Association with Elevated Ornithine Decarboxylase Activity and Formation of 8-hydroxydeoxyguanosine in the Liver. *Jpn J Cancer Res.* 88, 1149-1154.
- Wanibuchi, H., Yamamoto, S., Chen, H., Yoshida, K., Endo, G., Hori, T., Fukushima, S., 1996. Promotion Effects of Dimethylarsinic Acid on N-butyl-N-(4-hydroxybutyl) nitrosamine-induced Urinary Bladder Carcinogenesis in Rats. *Carcinogenesis*. 17, 2435-2439.

- Wei, Li., Wanibuchi, H., Salim, E.I., Yamamoto, S., Yoshida, K., Endo, G., Fukushima, S., 1998. Promotion of NCI-Black-Reiter Male Rat bladder Carcinogenesis by Dimethylarsinic Acid an Organic Arsenic Compound. *Cancer Letters*. 134, 29-36.
- Wei, M., Wanibuchi, H., Yamamoto, S., Wei, Li., Fukushima, S., 1999. Urinary Bladder Carcinogenicity of Dimethylarsinic Acid in Male F344 Rats. *Carcinogenesis*. 20, 1873-1876.
- Welch, A. H., Lico, M. S., Hughes, J. L., 1988. Arsenic in Ground Water of the Western United States. *GROUND WATER*. 26, 333-348 May-June.
- Winship, K. A., 1984. Toxicity of Inorganic Arsenic Salts. *Adverse Drug Reactions and Acute Poisoning Reviews*. 3, 129-160.
- Yamamoto, S., Konishi, Y., Matusuda, T., Murai, T., Shibata, M.A., Matsui-Yuasa, I., Otani, S., Kuroda, K., Endo, G., Fukushima, S., 1995. Cancer Induction by an Organic Arsenic Compound, Dimethylarsinic Acid (Cacodylic Acid), in F344/DuCrj Rats After Pretreatment with Five Carcinogens. *Cancer Research*. 55, 1271-1276.
- Yamamoto, S., Wanibuchi, H., Hori, T., Yano, Y., Matsui-Yuasa, I., Otani, S., Chen, H., Yoshida, K., Kuroda, K., Endo, G., Fukushima, S., 1997. Possible Carcinogenic Potential of Dimethylarsenic Acid as Assessed in Rat *in vivo* Models: a Review. *Mutation Research*. 386, 353-361.
- Yamanaka, K., Hoshino, M., Okamoto, M., Sawamura, R., Hasegawa, A., Okada, S., 1990. Induction of DNA Damage by Dimethylarsine, a Metabolite of Inorganic Arsenics, is for the Major Part Likely Due to its Peroxyl Radical. *Biochem. Biophys. Res. Commun*. 168, 58-64.
- Yamanaka, K., Katsumata, K., Ikuma, K., Hasegawa, A., Nakano, M., Okada, S., 2000. The Role of Orally Administered Dimethylarsinic Acid, A Main Metabolite of Inorganic Arsenics, In The Promotion And Progression of UVB-induced Skin Tumourigenesis in Hairless Mice. *Cancer Letters*. 152, 79-85.
- Yamanaka, K., Mizol, M., Kato, K., Hasegawa, A., Nakano, M., Okada, S., 2001. Oral Administration of Dimethylarsinic Acid, a Main Metabolite of Inorganic Arsenic, In Mice Promotes Skin Tumourigenesis Initiated By Dimethylbenz(a)anthracene with or without Ultraviolet B as a Promoter. *Biological Pharmacy Bulletin*. 24, 510-514.
- Yamanaka, K., Ohba, H., Hasegawa, A., Sawamura, R., Okada, S., 1989. Mutagenicity of Dimethylated Metabolites of Inorganic Arsenics. *Chem.Pharm.Bull*. 37, 2753-2756.
- Yamanaka, K., Ohtsubo, K., Hasegawa, A., Hiroyuki, H., Ohji, H., Kanisawa, M., Okada S., 1996. Exposure to Dimethylarsinic Acid, A Main Metabolite of Inorganic Arsenics, Strongly Promotes Tumourigenesis Initiated By 4-nitroquinoline 1-oxide in the Lungs of Mice. *Carcinogenesis*. 17, 767-770.
- Yamanaka, K., Okada, S., 1994. Induction of Lung-specific DNA Damage by Metabolically Methylated Arsenics via the Production of Free Radicals. *Environ Health Perspect*. 102 Suppl 3, 37-40.
- Yeh, S., 1973. Skin Cancer in Chronic Arsenicism. *Hum.Pathol*. 4, 469-485.
- Zhao, P.W., 1963. Report of Arsenic Poisoning Accident in Dan Zai Mercury Mine. Collection of Sanitation and Antiepidemic Material (Antiepidemic Station of Guizhou Province, China) (in Chinese).
- Zheng, B.S., Yu, X. Y., Zhang, J., 1994. The Geochemistry of Arsenic with Health and Diseases. (in Chinese).