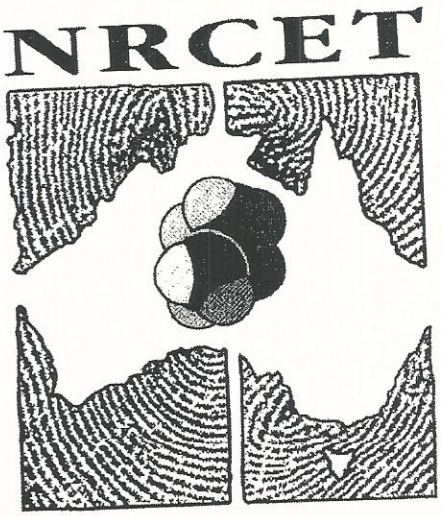
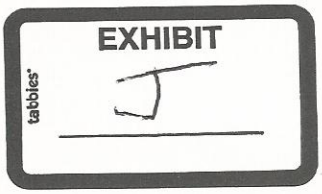
EXAMINATION OF THE POTENTIAL EXPOSURE OF ROYAL AUSTRALIAN NAVY (RAN) PERSONNEL TO POLYCHLORINATED DIBENZODIOXINS AND POLYCHLORINATED DIBENZOFURANS VIA DRINKING WATER



A REPORT TO THE DEPARTMENT OF VETERAN AFFAIRS, AUSTRALIA

THE NATIONAL RESEARCH CENTRE FOR ENVIRONMENTAL TOXICOLOGY (ENTOX)

QUEENSLAND HEALTH SCIENTIFIC SERVICES (QHSS)



EXAMINATION OF THE POTENTIAL EXPOSURE OF RAN PERSONNEL TO POLYCHLORINATED DIBENZODIOXINS AND POLYCHLORINATED DIBENZOFURANS VIA DRINKING WATER

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	Hexchlorodibenzodioxin
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

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

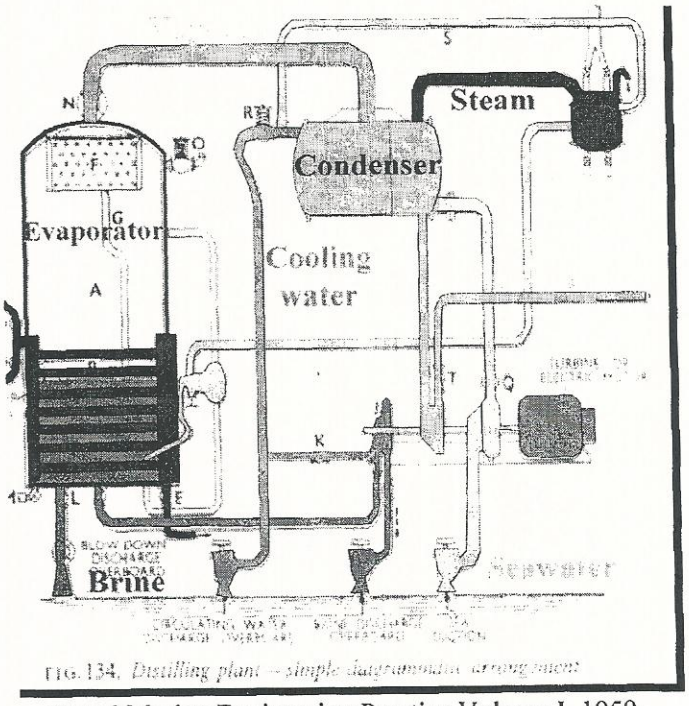
For the purpose of this study a commercially available rotary evaporator (Büchi, Switzerland) was used. Discussions with seamen and mechanics who served on RAN vessels during the conflict made certain that the principles by which solvents are evaporated in rotary evaporators were essentially the same 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

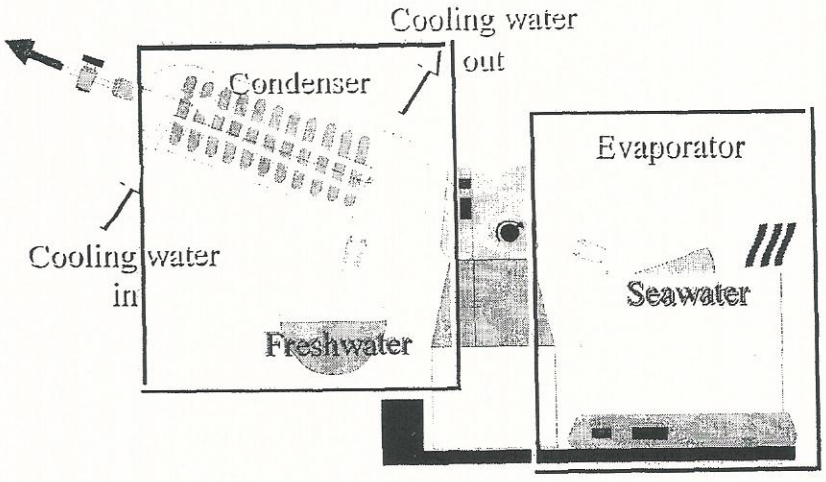


Figure 2: Schematic representation of the laboratory distillation unit

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
Heptachlorepoxide	-	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 58^0 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 (10^0C) 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

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.

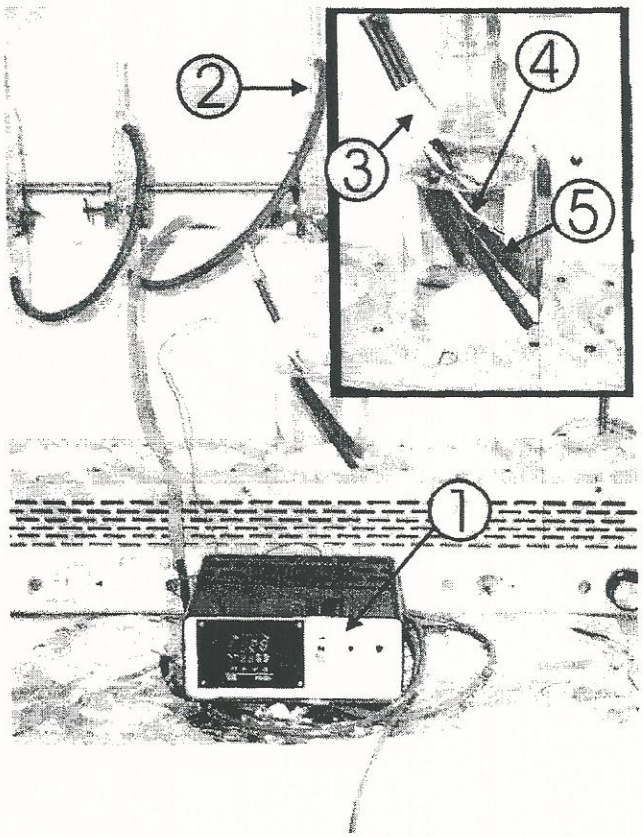
For Experiment 2 it was decided to include a clean-up step using H_2SO_4 and KOH impregnated silica gel in series in a Pasteur pipette. Samples were eluted using hexane, the hexane was evaporated and the samples were transferred into vials, concentrated and filled with toluene as described above.

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

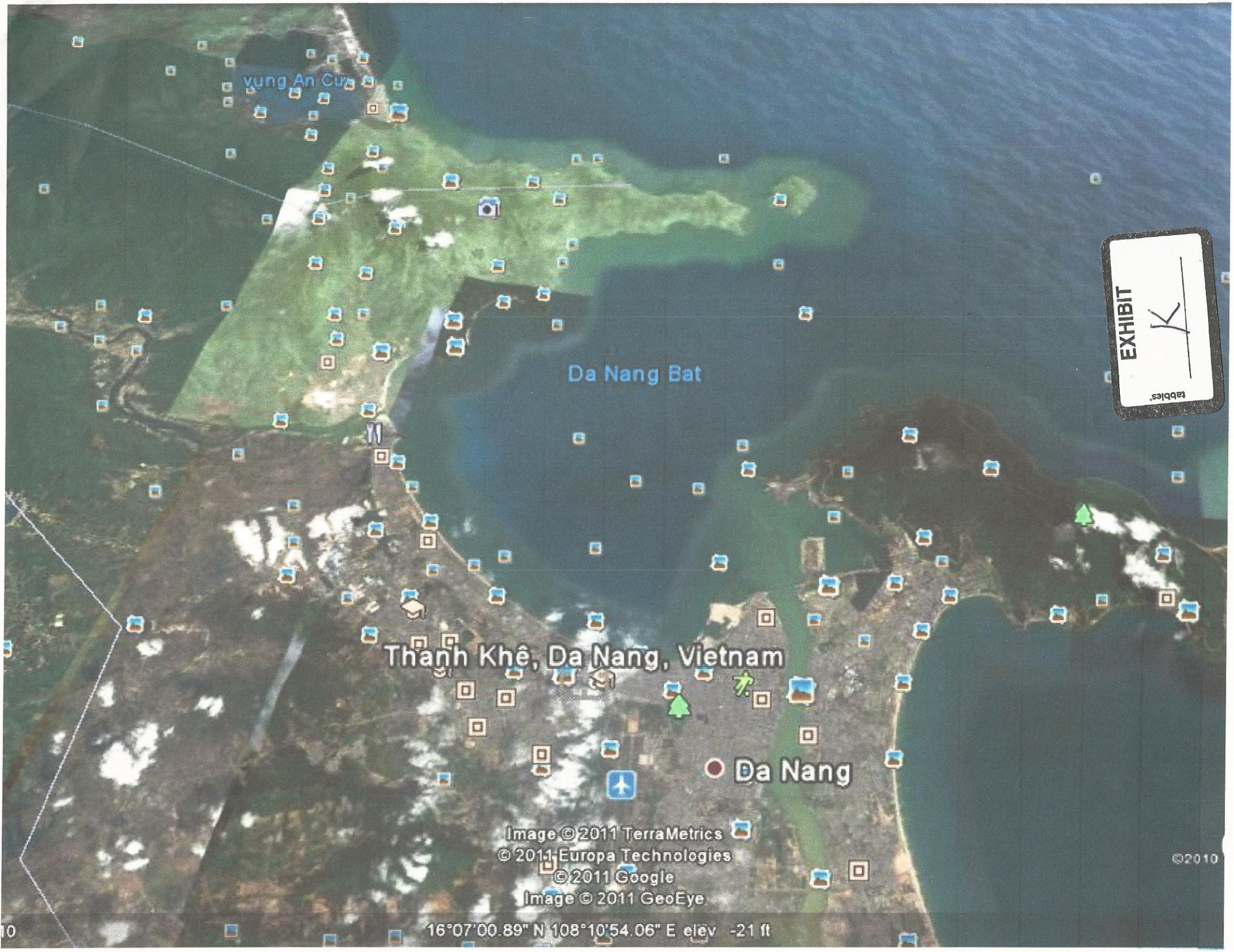
In order to evaluate "de-novo" formation of dioxins in the distillation unit itself from precursors, a system was developed in which an electrical heating element was inserted into copper tubing to represent the heating element in the distillation units aboard ships which also consisted of copper tubing (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.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