Item ID Number 01484

Author

Corporate Author

Report/Article Title Typescript: Statement of Dr. Alastair Hay

Journal/Book Title

Year 0000

Month/Day

Color ☐

Number of Images 14

Description Notes

STATEMENT OF DR. ALASTAIR HAY

Qualifications and background

1. I was born in Glasgow, Scotland, in 1947 and I currently reside in Leeds, England.

2. In 1969, I was awarded the degree of Bachelor of Science with Honours in Chemistry and in 1973 I was awarded a Doctorate of Philosophy in Biochemistry from the same university. From September 1972 until November 1977, I was a Research Fellow of the Zoological Society of London and from December 1977 until March 1979, I was a Research Fellow of the Department of Animal Physiology and Nutrition of the University of Leeds, England. During 1983 I was invited to join the Peer Review Panel of the Office of Health and Environmental Assessment of the Environmental Protection Agency (E.P.A.), U.S.A. and I participated in the preparation of the report entitled "Health Assessment Document for Polychlorinated Di-benzo-p-dioxins". I am presently Lecturer in Chemical Pathology at the University of Leeds, England, a position which I have held since April 1979. In 1978, I was invited to participate in an International Agency for Research in Cancer meeting in Lyons (France) to review the toxicity of the polychlorinated dibenzodioxins and dibenzofurans and to make recommendations for future studies.

3. My publications consist of some 50 referreed papers which have been published in many scientific journals, two books, one of which was The Chemical Scythe: Lessons of 2,4,5-T and Dioxin, and some 80 other scientific article and book reviews. A list of my publications is annexed hereto.

4. I have undertaken research work in the areas of Vitamin D metabolism, mutagenesis, drug toxicity and kidney damage.

5. Since 1976, I have reported upon scientific issues concerning inter alia, dioxins, Vitamin D, 2,4,5-T and neurotoxins in many articles for the scientific journal Nature.

6. I have visited Vietnam on two occasions, firstly in 1977 as a "Nature Travelling Scholar" for the journal Nature, and secondly in 1983 when I was invited to attend and participate in the conference "International Symposium on Herbicides and Defoliants in War: the Long-term Effects on Man and Nature", held in Ho Chi Minh City. At this conference, I was invited to be "rapporteur" for the section upon Toxicology and Cytogenecity.

7. I have undertaken extensive study of the medical and scientific literature in respect of, inter alia, the phenoxyacetic acids and TCDD and I have attended and participated in various symposia upon these subjects.

8. TCDD - General

8-1 Chemical Properties

Like all chlorinated dibenzo-p-dioxins, the tetrachlorinat isomer 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is stable to heat, acids, and alkali. TCDD is virtually insoluble in water (2×10^{-4} ppm), only slightly soluble in fats (44 ppm in lard oil), and more soluble in hydrocar (570 ppm in benzene), and at its most soluble in chlorinat organic solvents(1400 ppm in ortho-dichlorobenzene)[1].

8-2 Degradation by Microbial Action

Few microorganisms capable of degrading TCDD have been observed. Of 100 microbial strains examined, all of which are known to degrade persistent pesticides, only five exhibited any ability to detoxify TCDD. And in these strains, the efficiency of dioxin degradation was low, with manipulation of the culture conditions resulting in no improvement in efficiency[2]. The rate of degradation of TCDD in soil samples would appear to be a function of the type of microorganisms present and the concentration of th dioxin. The half-lives of TCDD in soil from Utah and Florida were 320 and 230 days, respectively[3]. The dioxin half-life at Seveso is reported to be of the order of 2-3 years[4] and possibly longer.

9. Toxicological properties of TCDD in animals

9-1 Distribution in Body

From the few reports available on the tissue distribution and excretion of TCDD, it is evident that the chemical is rapidly but incompletely absorbed from the gut[7-10]. Because of its partition coefficient - the dioxin is more soluble in fats than aqueous solution - TCDD tends to be stored in fatty tissue. The liver is the principal storage site for TCDD in the rat[7] and guinea pig[11].

Other species where TCDD has been observed include fish, cattle, rhesus monkeys, and humans. Spotted sun fish taken from a 2,4,5-Tsprayed area in Florida were recorded as having 4,4,18 and 85 ppt of TCDD in skin, muscle, gonads, and gut, respectively[12]. Ranges of TCDD from 20 to 60 ppt have been reported in a small percentage (3.5%) of samples of beef fat taken from cattle known to have been exposed to 2,4,5-T [13,14]. No detectable levels of TCDD were observed in the livers of these animals[13].

Accumulation of TCDD in the rhesus monkey occurs in the skin, adipose tissue, and muscle after oral dosing[15]. As far as humans are concerned TCDD has been detected in human breast milk; levels of TCDD from 40 to 50 ppt were measured in samples collected from women residing in areas in Vietnam heavily sprayed with the herbicide Agent Orange in 1970 and analyzed 4 years later[16]. Samples of breast milk obtained from mothers residing in 2,4,5-T sprayed areas in the United States had no detectable levels of TCDD - minimum detection level 10 ppt[17]. Levels of 3-57 ppt of TCDD have also been measured in fat tissue taken from 33 former Vietnam War veterans[18]. Curiously, measurable levels of dioxin were also detected in fat from ten individuals in the matching control group[18].

1€ = Bar Course

9-2 General Toxicology

Pronounced weight loss and anorexia are common findings in most animals fed lethal concentrations of TCDD[19,20-22]. Atrophy of the thymus is a constant finding in all animals given a lethal dose of the chemical[19, 23,20,24,25]. According to Vos et al.[26], sublethal doses of TCDD, 2-5 ug/kg body weight, affect the lymphoid system in the rat causing a suppression of cell-mediated immunity. The offspring of pregnant rats fed sublethal doses of TCDD on days 14 and 17 of gestation and postnatally on days 1, 8 and 15 had a lowered lymphocyte count in the thymus cortex. This fall resulted in the impairment of cellular immunity and an increase in the time taken to reject allografts[26].

The depression in thymus-dependent immune function may explain the observation of Greig[27] that many rats poisoned with TCDD die with severe lung infection. But as Greig points out, this in itself is not sufficient to explain the toxic action of TCDD since deaths have occurred in both TCDD-treated germ-free and SPF rats maintained in sterile conditions[28]. It is known that the resistance of rats to bacterial infections with Salmonella is reduced by TCDD[29]. But Vos[30] believes that this may be due to endotoxin present in the Salmonella - TCDD-treated mice show a marked increase in susceptibility to endo toxin.

The immunosupressuve effect o f TCDD is not restricted to rats which are considered to be poor immunologic responders: the effect occurs equally in rats which are good immunologic responders[31]. Although TCDD's mechanism of induction of immunosuppression is unknown, it is clear that the effect is reversible, at least in mice which are good immunologic responders[31].

9-3 Hepatotoxicity

The liver is a major target organ for TCDD and is severely affected by the chemical. TCDD will cause extensive necrosis of the liver in rabbits[34-5], but a more localized focal centrolobular necrosis in rats[20,24,27,32,33]. The size and shape of hepatocytes in rats show considerable variations, and large multinucleated hepatocytes and nuclear enlargements have also been observed[23,32]. Electron microscope studies have shown that the multinucleate cells arise by fusion of parenchymal cells[33]. According to Greig[27], the formation of those abnormal cells appears to be the result of changes at the cell membrane soon after dosing. Histochemical studies show that ATPase activity disappears rapidly[36-7], indicating that the parenchymal cell membrane is a target site for the toxic action of TCDD.

The loss of ATPase is also reported to have been observed in biochemical studies of isolated membrane preparations[27].

9-4 Edema

Mammalian species and chickens exposed to TCDD develop edema. Rat fetuses treated prenatally with the chemical developed subcutaneous edema in the head, neck and trunk[38]. Severe terminal edema has been observed in about a quarter of mice receiving a lethal dose of the chemical[39,26]. The edema occurred in subcutaneous tissue and in abdominal and thoracic cavities. Primates, too, developed edema after TCDD intoxication. The edema noticeable in the lips, is accompanied by reduced serum albumin levels[40,41].

9-5 Less Specific Toxic Effects

TCDD causes a variety of other lesions in different animal species. Damage to kidney tubular epithelial cells occurs in rats given lethal doses of the chemical[32].

Ulceration and necrosis of the glandular section of the stomach[40], chloracne, loss of eyelids, facial alopecia and abnormal growth and loss of toe and finger nails also occur in monkeys exposed to TCDD[41]. Testicular atrophy has been described in both primates[40]and rats[24].

Horses exposed to TCDD experienced loss of hair, chronic emaciation, skin lesions, edema, intestinal colic, conjunctivitis, and joint stiffness.

9-6 Teratogenic Effects

A chemical is a teratogen when it causes developmental disturbances in the embryo which result in congenital malformation. If a chemical kills the embryo, it is said to be embryocidal, and if it produces tissue damage (not necessarily resulting in malformation) it is embryopathic. The term embryotoxic generally refers to any harmful effect on the embryo.

Most of the teratogenic and embryotoxic studies involving TCDD have been conducted using the herbicide 2,4,5-T[42]. Various formulations of the herbicide with a TCDD content of 0.02-30 ppm have been shown to be teratogenic for mice[43-4].

TCDD causes terata in the C57 Bl/6 strain of mice at a single dose of 1 ug/kg when given on day 10 of gestation[45]. In other mice strains, doses

of 1-3 ug/kg administered on days 6-15 of gestation will cause kidney abnormalities and cleft palate in most animals. (See Table 1 for details). It is also clear from the results available that there is a difference in sensitivity to TCDD between strains of mice. For example, 3 ug/kg of TCDD on days 6-15 of gestation is required to produce cleft palates in the NMRI mouse strain[42], whereas 1 ug/kg over the same period will have this effect in the CD-1 strain[46].

The proportion of fetuses born malformed increases as the TCDD dose increases as shown by the differences between the dose which will produce a teratogenic effect and the corresponding LD_{50} dose (the concentration required to affect 50% of the animals)[42-44]. Repeated daily oral doses of TCDD from 25-400 ug/kg had an increasing fetotoxic and teratogenic effect in mice with some 97% of the animals affected at the highest dose[47].

10. Toxic Effects in Humans of TCDD

There have been 24 recorded accidents in chemical plants manufacturing trichloro and pentachlorophenol and the information in the published and unpublished literature shows quite clearly that TCDD is toxic to humans.

The clinical effects on humans resulting from these accidents show that the symptoms of TCDD poisoning of humans include the following:

(a) Skin changes: chloracne, hyperpigmentation, hirsutism.

(b) Systemic effects: liver damage (mild fibrosis); raised serum hepatic enzymes, glutamic oxaloacetic transaminase (SGPT); increased excretion of porphyrins in urine; disorders of fat metabolism (hypertriglyceridemia, hypercholesterolemia) and carbohydrate metabolism; cardiovascular, urinary tract, respiratory, pancreatic, and digestive disorders (flatulence, nausea, vomiting, diarrhea); loss of appetite and weight loss; muscular aches and pains, and pain in joints; reduced primary immune capability.

(c) Neurological effects: polyneuropathies; lower extremity weakness; impairment of sensory functions including sight disturbances, loss of hearing, taste, and sense of smell; headaches.

(d) Psychiatric effects: depression, loss of energy and drive, disturbance

of sleep, uncharacteristic bouts of anger.

Chloracne is a sign or symptom of dioxin poisoning but the absence of chloracne does not rule out exposure or the possibility of long-term effects, as shown by Oliver's and Hardell's works.

11. Mutagenicity and carcinogenicity of TCDD

11-1 There is no doubt about the carcinogenicity of TCDD in animals. Several reports have shown unequivocally that this chemical will cause cancer in rats and mice (see Table 2 for summary). The liver, lung and thyroid appear to be the organs in which tumor development is most common.

11-2 In view of TCDD being a carcinogen in animals the possibillity exists that it is a potential carcinogen in humans. There is evidence in the literature that exposure to TCDD and/or phenoxyacetic acids can cause an increase in soft tissue sarcomas and gastrointestinal cancer.

11-3 There is a widespread belief among cancer workers that DNA damage is involved in the induction of cancer[48]. A chemical which will damage DNA and cause a cell to mutate is classified as a mutagen. Assay systems, commonly employing bacterial, fungal, or mammalian cell lines, have been developed to detect mutagens. Chemicals which are carcinogens in animals are usually identified as mutagens by these assays, usually referred to as short term tests[48-50]. However, not every mutagen will cause cancer in animals. The fact that most mutagens probably do cause cancer has enabled the tests to be used to identify potential carcinogens. A recent international study has confirmed that there are short-term tests that can be used to predict carcinogenic activity[51]. All tests give false negatives and false positives too, and it is well known that tests give better results for one class of chemical than another. However, it is possible to select tests which are complementary and to include these in a battery of tests which will identify most potential carcinogens[51].

Most chemicals require metabolic activation before they are mutagenic. In mammals this activation is performed by microsomal enzymes in the liver. Most tests now employ, therefore, a liver microsomal preparation, the so-called S-9 mix, as an obligatory activation step[52].

11-4 TCDD has been tested for mutagenicity using the in vitro short-term tests in both the presence and absence of the S-9 mix. Two groups have reported

that when TCDD was tested in the Ames bacterial test using the Salmonella typhimurium strain TA 1532 without the S-9 mix, a positive result was obtained, suggesting that the chemical was indeed mutagenic[53,54]. TCDD is also reported to be mutagenic in the Escherichia coli strain Sd-4[53]. The TA 1532 strain which measures the reversion of the cell line from a dependence on histidine to independence (the strains are placed on medium without histidine and hence will only grow if they mutate and become independent of histidine, the factor limiting growth) is well known as a strain which detects frame shift mutagens and hence chemicals which cause mutagens by intercalating with DNA[55]. The chemicals insert themselves into DNA, between the base pairs, thus causing a partial unwinding of the DNA double helix. This effect might lead to a misreading of the genetic code during replication of the cell, and thus to mutation.

A positive result for TCDD with the strain TA1532 has not been obtained in every laboratory, however. McCann[56] has used TA 1532 as well as strains TA 1535, TA 1537 and TA 1538 with and without the S-9 mix, but without success: negative results were obtained with all the strains. TA 1537 and TA 1538 are sensitive indicators of frameshift mutagens and have replaced strain TA 1532 in most laboratories. It is all the more surprising therefore, that if TCDD is an intercalating agent, it fails to give a positive result with the strains optimised to detect this. Other workers also report negative results for TCDD tested with strains TA 1535 and TA 1538[57]. Recent unpublished work also confirms that the chemical is negative with strain TA 1537[58].

11-5 Thus the evidence which would implicate TCDD as a mutagen in the Ames test is confusing. It is not at all clear why the early results should give positive readings and why those post 1976 should all be negative. A majority of investigations find that TCDD is not a mutagen in the Ames test. However, a negative finding in the test does not mean that the chemical has no mutagenic properties. It is well known that no single short-term test will identify all potential carcinogens as mutagens, every test has a number of false positives and false negatives[51].

Because TCDD will cause cancer in test animals it was clearly necessary to find some explanation for its ability to do this. One avenue of investigation was to see whether TCDD could be identified as a potential carcinogen, using another short term test.

11-6 The one selected was a mammalian in vitro test using baby hampster kidney cells in a cell transformation assay[59]. This work was carried out by

colleagues of mine, Drs. John Ashby, J.A. Styles and B.M. Elliot at Imperial Chemical Industries, Central Toxicology Laboratory, Macclesfield, England. The cell transformation assay, using baby hampster kidney cells (fibroblast-like morphology) in a semi-solid agar medium, has been validated in a study using 120 chemicals; the test performed well and was found to be capable of discriminating between carcinogens and non-carcinogens with about 90% accuracy. Care is necessary in the interpretation of results from short term tests since these can be altered by the choice of chemicals[53]. The most reliable approach is to include related carcinogenic and non-carcinogenic structural analogues of the test chemical in the assay at the same time. Only if the control analogues give the correct responsewill the result for the unknown be reliable; if the controls give an incorrect response, the assay will be invalid. The procedure for the cell transformation assay is described by Styles[59] and is outlined in Figure 1. Several dioxin isomers were used in the assay and all were dissolved in the solvent dimethylsulfoxide (DMSO); the solvent has no effect on cell transformation.

In the test, TCDD gave a clear, positive response- shown in Figure 2. A pure and impure preparation of TCDD were tested in this assay; both gave positive results, a finding which indicated that the impurity had no effect on the test result. The unsubstituted dibenzo-p-dioxin (DBD) a non-carginogen in animals [60] used as the structually appropriate negative control chemical was negative in this assay, a finding also seen with a number of Salmonella strains.[61] Octachlorodibenzo-p-dioxin (OCDD), a compound not tested for carcinogenicity in animals was also negative in the cell transformation assay (see Figure 2). Two other isomers 2,8-dichlorodibenzo-p-dioxin and 1,3,7-trichlorodibenzo-p-dioxin were both weakly positive in the assay (see Figure 3).

Although it would have been preferable to have tested the 2,7-dichloro isomer - a weak carcinogen in male B3C3F1 mice [62] the 2,8-dichlorodibenzo-p-dioxin dompound which was tested is a closely related analogue. The result that was obtained for the 2,8 dichloro isomer suggests that this too may be a weak carcinogen. As 1,3,7 - trichlorodibenzo-p-dioxin gave a weak reproducible positive result in the BHK assay it too may have carcinogenic properties. The response obtained with the 1,3,7-trichloro isomer was lower than the 2,8-dichloro isomer suggesting that the chlorine atom at the peri position - C-1 on the dibenzo-p-dioxin ring - may affect its toxic properties. This would seem to be borne out by the negative response obtained with the fully chlorinated octachlorodibenzo-p-dioxin where all four peri positions (C-1, 4, 6 and 9) are occupied.

The positive response obtained with TCDD in the BHK assay is in agreement with the animal data identifying the chemical as a carcinogen.

11-7 Even though it may not be clear whether TCDD is causing mutation of the gene or chromosomal damage in the BHK cell line to cause cells to transform there is other recent evidence to indicate that the chemical has mutagenic properties. In the D_7 strain of the yeast Saccharomyces cerevisiae TCDD induced both point mutations and mitotic gene conversion when the chemical was assayed after metabolic activation with a liver preparation [63]. The frequency of events increased in a dose dependent fashion with increasing concentrations of TCDD. Mutation and mitotic gene conversion did not occur when the activation system was excluded from the tests. In contrast TCDD is said to be a direct acting mutagen in the L5178Y mouse lymphoma cells [64]. Tested without activation, TCDD induced mutation in 3 selected cell systems, those using methotrexate, excess thymidine and thioguanine respec tively. In two other tests where the L5178Y cells were used with ouabain and cytosine arabinoside, TCDD did not induce mutations. In view of its action on the mouse lymphoma cells the authors, Rogers, et al [64] say that TCDD is a direct acting mutagen and they note that previous authors [53,55] have suggested that the chemical may exert its mutagenic effect through intercalation with DNA.

11-8 Where intercalation occurs the properties of both the che mical and the DNA are altered in a standard way. For example, the melting temperature of DNA is increased by up to 5-10°C, and the visible spectrum of the chemical is altered, undergoing a bathochromic (red) shift. In the case of the intercalating agent, Ellipticine, following intercalation the rise in the DNA melting temperature was 8.8°C, and the bathochromic shift about 40 mm [65]. When we tested TCDD as a potential intercalating agent with calf thymus DNA it had no effect on the melting temperature, and no bathochromic shift was observed [66]. On the basis of these findings it would appear that TCDD is not an intercalating agent.

11-9 If TCDD does not intercalate with DNA then the question remains: How does it act inside the cell to bring about the mutagenic and cell transforming events described above?
Poland, et al [67] recently presented results demonstrating the tumour promoting properties of TCDD. These were said to be sufficient to explain the carcinogenicity of TCDD in rodents [68-71] since the chemical appeared to have no carcinogenic initiating activity as evidenced by its limited ability to interact with DNA [72] or to mutate the usual tester strains of Salmonella tryphimurium in the Ames test [55,56,73-76]. The definition of a promoting as opposed to an initiating agent

is far from clear [77] - most promoters do not have genotoxic, chromosome - altering, or cell-transforming properties. TCDD, however, has all of these [66,63,64], and for this reason it cannot yet be defined purely as a promoting agent.

11-10 The importance of gene-mutation vs. chromosomal changes in the initiation of cancer was reviewed recently by Cairns [78] who suggested that an agent's ability to cause chromosome rearrangements may be of far greater importance as a cause of cancer than is its gene-mutagenic properties. TCDD is currently recorded as only having a weakly positive chromosone damaging effect in the bone marrow of rats [84] and mice [79]. ON the other hand TCDD has a powerful inhibitory effect on mitosis in the African Blood Lilly [80] resulting in the formation of dicentric bridges and chromatid fusion and cells with multinuclei or a single large nucleus. Multinucleated cells have been observed in mammals following treatment or poisoning with TCDD [20,28,81,82].

Whether or not TCDD's apparent clastogenic properties are sufficient to initiate cancer remains to be seen. It is important to remember, however, that there are many different ways in which chemicals can cause mutations.

11-11 Given the data that is available, it is fair to say that while the evidence in the Ames test is equivocal, other tests indicate that TCDD could be mutagenic, which suggests that it may also have the properties of an initiater. Thus, it may yet be shown to be a carcinogen.

11-12 Today, the most widely held theory of carcinogenesis utilizes the concept of oncogenes. Every human cell contains 23 pair of chromosomes on which the genes are found. It is believed that each chromosomal pair contains oncogenes, which, if expressed or activated, will cause a cell to become malignant. Certain chemicals and viruses are known to be oncogene activators. One of these chemicals is 3-methyl cholanthrene. This chemical has very similar properties and toxicological effects to TCDD. It is therefore possible that TCDD initiates cancer as an oncogene activator.

12. Effects on fertility

The fact that TCDD has a weak chromosome-breaking effect in rat bone marrow cells, [84] and that the chemical reduces spermatogenesis in rats, [85] and testicular DNA synthesis in mice, [86] suggests that DNA in germ cells may be affected by the chemical. Direct effects on fertility have also been observed. Male and female rats in a three-generation study fed TCDD at 0.01 ug/kg had a lower survival rate, reduced growth rate and smal ler litter size. [87] Primates fed TCDD at the parts-per-trillion level have irregular menstrual cycles, excessive

hemorrhaging during menstruation, a reduced conception rate, and high incidence of early abortions.[88]

13. Conclusion

Since TCDD has been shown not only to be neurotoxic, immunosuppressive, hepatotoxic and to have effects on fertility, but also may be a complete carcinogen; there is no known safe level for any human intake of 2,3,7,8-TCDD. That is, there is no "no effect level" for TCDD.

14. TCDD in Agent Orange

It is generally believed that the average concentration of TCDD in Agent Orange was 2 ppm, calculated as a weighted mean [89].

Documents which have recently come to light show that this is, in fact, a very conservative figure. The concentration of TCDD in some batches of the trichlorophenol manufactured by Monsanto Chemical Company, and subsequently converted to 2,4,5-T for use in Agent Orange between 1965 and 1968 in Vietnam, ranged from 10 to 40 ppm. On the basis of this evidence, the suggestion tha t the average TCDD contamination of Agent Orange was 2 ppm is a very conservative estimate. In fact, it was probably much higher.

15. EPA Assessment Panel

In 1983 I was a member of an expert panel set up by the Office of Health and Environmental Assessment of the E.P.A.(USA) to review reports to be released for public comment, on levels of TCDD in waste, air and water.

This panel came to the conclusion that TCDD is one of the two most potent carcinogens ever evaluated by the E.P.A.. The other was a mixture of two hexachlorodibenzodioxin isomers, contaminants of pentachlorophenol.

16. SEVESO

From the examination of 34 foetuses from Seveso women who had experience spontaneous or induced abortions, it was found that only one had an identifiable defect, namely Down's Syndrome. The International Steering Committee of the Lombardy Region concluded that this finding does not necessarily mean that TCDD is not harmful to the human embryo. The Committee believes, and I quote from their 6th Report, that the "more likely" explanations would be one of the following:-

" (2) Harm was not detected because the women with the highest exposure yet experiencing abortions or having malformed foetuses were not detected.

(3) the risk of adverse reproductive outcome from exposure to TCDD was so low that insufficient women were exposed to allow detecting the adverse effect.

(4) pregnant women were not exposed to sufficiently high doses to cause the adverse effects."

17. 2,4-D

17.1 In my opinion, 2,4-D is neurotoxic to humans. There are a number of reports in the scientific and medical literature which describe neurotoxic symptoms as a result of agricultural and industrial exposure to 2,4-D.

17.2 Hardell's studies show that exposure to 2,4-D is also associated with soft tissue sarcoma.

Exposure to 2,4-D and/or MCPA gives a four-fold increase in risk of developing soft tissue sarcoma[90].

18. 2,4,5-T

18.1 Animal studies show that 2,4,5-T is a teratogen, even when there is no detectable contamination by TCDD therein. The concentration of 2,4,5-T required to cause malformation is generally three (3) orders of magnitude higher than TCDD.

18.2 One of Hardell's studies shows that where exposure to dioxin and phenoxy acids has occurred the risk factor of developing S.T.S. increases to 17 [90].

19. Hardell's Studies

In my opinion, Hardell's work is scientifically valid and produces significant results. He has taken account of criticisms of his original research by repeating and verifying his first findings. These findings still stand, namely that exposure to phenoxyacetic acids increases the risk of developing soft tissue sarcoma and malignant lymphoma.

20. The International Symposium on Herbicides and Defoliants in War

20.1 A discussion of the conclusions of this conference can be found in my report for the journal, Nature, "Defoliants in Vietnam: The Long-term Effects" which is annexed hereto.

20.2 The conference concluded that more investigation was needed in respect of liver cancer, in particular to ascertain the contributions of hepatitis B and aflatoxin to the rate of liver cancer in Vietnam.

20.3 The work of Dr. Can (Institute of Protection of Mother and Newborn in Hanoi) in the area of reproductive toxicology was considered to be well-conducted. This study found a significant increase in unfavourable pregnancy outcomes amongst the unexposed wives of exposed husbands. Unfavourable pregnancy outcomes were defined as stillbirths, miscarriages, malformed children or babies which were small for date.

20.4 After returning from the conference, I submitted the cytogenetic study of Dr. Cung Binh Trung(Department of Health, Hanoi) to several cyto-geneticists at the University of Leeds. Dr. Trung's study showed a significant increase in sister chromatid exchanges in the peripheral lymphocytes of exposed Vietnamese subjects compared with unexposed Vietnamese controls. My colleagues, having examined his methods, found the study to be well-conducted and the conclusions to be scientfically valid. Both Dr. Trung's work and an assessment of it will appear in the proceedings of the conference to be published by the Stockholm International Peace Research Institute (SIPRI). The proceedings are being edited by Dr. Arthur Westing of SIPRI.