

**ORAL SUBMISSION for 1080 application number : HRE05002**  
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**“ I ngā wā o mua” – The past informs the present.**  
**“ Foresight should be sought as hindsight is dearly bought”**

I initially completed a Bachelor of Science degree at Victoria University in Physiology and currently I am an Accident and Medical Practitioner. I apologize for using such a low-tech overhead projector but this level of technology corresponds to that used by Eason & colleagues in his developmental (1999) and sub-chronic sub-lethal toxicity 1080 studies ( 2002) that initially drew my interest to the topic of 1080, studies which I did not think were very good, in fact, I could have done them when I was an undergraduate science student 25 years ago! I initially knew absolutely nothing about 1080 and was totally indifferent to its use until the issue was raised by a patient late last year.

(\* In the Graf boys DVD *A Shadow of Doubt* –I deliberately chose to be interviewed in a local Primary School with a playground in the background, the level of regulatory science for 1080 and recommendations by the ERMA advisory report relative to the complexity of the real world and insights from the most recent research, is that of a Primary school level, and the derivations ( Forunda et al, 2007) of such calculations for NOAELS/Benchmark dose responses/TDIs ( based on Eason’s et al (1999, 2002), work corresponds well to the artificial “constructs” of a playground as opposed to the real 1080 hazards listed below for young children in rural New Zealand ).

My greatest concern is with regard to the absence of research for episodic sub-lethal and chronic sub-lethal exposures to vulnerable human populations, such as the growing child in the womb, lactating or breastfed infants, young children, and adults with medical conditions, especially impaired renal failure.

My first deliberations about 1080 were regarding possible exposures that I as a non-environmental scientist thought needed to be addressed. On looking at the literature I was surprised that the obvious had not been looked at.

#### **LEVELS OF 1080 IN CERTAIN FOODS CONSUMED PREDOMINANTLY BY N.Z MAORI THAT NEED TO BE MEASURED.**

The results of field research undertaken to determine the levels of uptake and persistence of the toxin sodium monofluoroacetate (SMFA/ compound 1080)) from baits used to control the brush-tailed possum for the plants of two species, pikopiko (*Asplenium bulbiferum*) and karamuramu (*Coprosma robusta*), used by a Maori community for food and medicine, suggested negligible risk of humans being poisoned by consumption of such plants (Ogilvie *et al.*,2006). 1080 levels were detected only in karamuramu, at a maximal concentration of 5 parts per billion (ppb) 7 days post exposure with levels decreasing to 2.5 ppb after 14 days.

**\*Large text corresponds to the verbal content presented on 24/05/07; commentary in small text is not in verbal presentation but clarifies the significance of larger text concepts and corrects some of the outdated, simplistic, skewed and misleading scientific advice given in the Advisory Report & is in response to the Chairman’s request. This is a fuller statement that is part of a pending, formal peer-reviewed article.**

The authors state that “ ..to allay community concerns that minute concentrations of 1080 might influence the medicinal properties of plants, it is suggested a withholding period of 30 days after 1080 control operations...”

If researchers really wished to allay Maori community concerns then they would have evaluated the levels of 1080 for the two commonly consumed plants, Puha (sow thistle; *Sonchus sp.*) and watercress (*Nasturtium officinale, N.acquaticum*), which have been proposed as plausible cancer-protective dietary factors which may contribute to the lower incidence of colorectal cancer rates in Maori compared to non-Maori New Zealanders (Thomson & Shaw, 2002). Given that these two plants are eaten in significant amounts, by Maori and also some other Pacific Island people, the lack of any research pertaining to 1080 is a serious concern.

Furthermore, a recent field study for the chlorine containing compound perchlorate, indicated that plant accumulation for this contaminant from streams in watercress was substantial, with dry leaf concentrations up to two orders of magnitude greater than bulk water levels (Tan K *et al.*, 2004). As watercress is a floating aquatic plant with short roots that could potentially take up 1080 from surface water and shallower sediment pore water, the fate of 1080 in waterways, uptake and persistence, needs to be urgently studied, given high consumption rates by Maori, even if residual sampling of water levels have been reported as containing negligible (<1 ppb-3.5 ppb) or no 1080 at all (Eason *et al.*, 1999), as significant amounts may have been taken up already by the watercress.

Interest in elevated levels of arsenic in certain rivers and NZ aquatic systems have led to recommendations that watercress not be consumed from these areas (Robinson *et al.*, 2003). Studies for 1080 and watercress are long overdue.

Although no residue tissue levels of 1080 were found in a study of captive longfin eels when exposed to cereal baits, detectable levels were observed, which were a function of dose and frequency of feeding on contaminated possum muscle or gut tissue (Lyver *et al.*, 2005), hence non-captive eel testing ought also be done to check the field scale of 1080 tissue residues that may exist post 1080 operations and assure safety for those rural populations with no commercial interests, that may enjoy eel with their watercress.

Minute amounts of 1080 may be found in other aquatic food sources such as freshwater crayfish, however these amounts are not thought to pose any human health risk (Suren & Bonnett, 2006), and the authors reassure that “the potential risk to humans consuming crayfish containing 1080 is virtually non-existent, as an 85kg person would need to consume over 40kg of contaminated crayfish tails in a single sitting to receive an LD50 dose.

My concern is not with the large amount of 1080 calculated above that is needed to cause severe health problems or death but relates to a different issue, namely, **whether chronic or episodic exposures to sub-lethal or even very low doses (< 3.5 ppb) pose any health risks to humans?** What about the rural pregnant Maori woman who consumes large amounts of watercress after an aerial 1080 drop?

## MECHANISM OF TOXIC ACTION OF 1080

To understand potential risks to health that may be caused by a chemical then it is desirable to know how the chemical works and what adverse effects may result from this mechanism or other possible additional mechanisms on the host organism.

It is commonly thought that fluoroacetate, once absorbed, inhaled or ingested is converted within the body in subcellular structures called mitochondria, into a toxic metabolite fluorocitrate, which is both an inhibitor of and substrate for the mitochondrial aconitase enzyme, causing aconitase inactivation, resulting in citrate accumulation and decreased energy production in the form of ATP “cellular-currency”, due to Krebs cycle interference, which in turn leads to cellular energy deprivation and death. (Clarke 1991; Goncharov et al, 2006; Weaver 2003.)

(The crystal structure of the aconitase enzyme-inhibitor complex from the reaction of fluorocitrate with aconitase has been identified (Laube et al., 1996) but the authors noted that whether this complex did or did not explain the mechanism of 1080 toxicity remains unclear.)

Over two decades ago Kun (1982) provided evidence that erythro-fluorocitrate could bind to at least three mitochondrial inner-membrane proteins (also known as *transmembrane proteins*) and he believed that chemical modifications to them by fluorocitrate is the molecular basis of toxicity.

(*Transmembrane proteins* are analogous to CD/DVD slots/USB inputs/modems etc, which act as a physical interface to allow external messengers or information to get into a computer and activate, repress or modify certain programs within that computer. 1080 (the chemical message contained on a disc/CD/DVD; e-mail attachment etc) connects to and acts on the proteins that bridge the inner membrane or wall of the mitochondria, transmitting certain molecular instructions to the interior of the cells that cause many different effects/responses within the mitochondria. Different types of transmembrane proteins are the key to understanding how the same chemical/substance may have different biological effects.)

One of these transmembrane proteins involves the *citric acid carrier*, another binds to an iron-sulphur containing protein bonded to *glutathione* –the latter is important in detoxification systems and in anti-oxidant protection.

*Citric acid carrier* -Kun (1982) noted that nano-molar concentrations of erythro-fluorocitrate inactivate the transport of citrate in and out of mitochondria. Experimental interference of membrane citrate carrier proteins recently have been found to be associated with some important functions such as a regulatory role in glucose-stimulated insulin secretion (Joseph et al, 2006) who found inhibition of this citrate carrier protein leads to defective or impaired glucose-stimulated insulin secretion. This has implications for diabetes etiology and unlike the comments of the ERMA Appendices to the Evaluation and Review Report (henceforth called Advisory Report) B16.1.2 page 331 which notes that “reference to the term ‘1080-induced’ diabetes may give risk to misunderstandings about the impact of 1080 exposure. The Agency notes that the use of the term is of historical significance only.” Recent reviews by Maechler & de Andrade (2006) and Wiederkehr & Wollheim (2006) and perturbation of mitochondrial function in life’s earliest stages (Lee et al, 2005) which 1080 no doubt could achieve easily at sublethal doses, given the ultra-sensitivity of fetal endocrine systems, and the fact that patients with mitochondrial diabetes and a corresponding mouse model displaying defective glucose-stimulated insulin secretion (de Andrade et al, 2006) with overt diabetes exist, would indicate that the Agency Report’s scientific comments are of non-significant historical value.

Kun (1982) also noted that blocking the citric acid carrier may contribute to the neurotoxic effects of 1080 in those animal species dependent on the intra-mitochondrially generated citrate efflux or movement out of the mitochondria, for biosynthesis of the important brain neurotransmitter called acetylcholine, such inhibition of so called cholinergic centers in the brain have been poorly studied and will be discussed further below on mechanisms of possible neuro-developmental toxicity.

Interestingly a recent study ( Knauf et al, 2006) found a longevity gene called *Indy* ( for ‘ I’m not dead yet’) which encodes for dicarboxylate and citrate transport/flux across cell membranes, and decreasing INDY activity, leads to prolonging lifespan of flies. It would be interesting to note if 1080 inactivation of the mitochondrial citrate carrier protein has any detrimental effect on longevity gene activity.

**Glutathione**- represents the major low molecular weight chemical that has an important role in complex cellular processes including functioning in signaling of detoxification gene expression, cell signaling and protein function regulation (Biswas et al, 2006) and a central role in protecting against types of cellular stress [known as oxidative and reductive stress] the latter two processes which may be induced by 1080. Glutathione systems/levels may be overwhelmed or depleted in the fetus/very young much more easily than in adults, leading to cellular “stress” with the formation of harmful chemicals called reactive oxygen species which may have possible negative cellular consequences such as mitochondrial DNA mutation – see below.)

But what of the third type membrane-bound protein?

Our current knowledge of membrane bound proteins and **signal transduction** is of vital importance to understanding that fluoroacetate is much more than just a metabolic inhibitor (overhead of some membrane-bound protein and signal transduction systems.)

**Signal transduction** A basic process in molecular cell biology involving the conversion of a signal from outside the cell to a functional change within the cell. An external or primary chemical signal (such as a hormone or neurotransmitter) interacts with a receptor or receptor –complex if more than one protein is involved, on the cell surface; this interaction causes a change in a second messenger (such calcium or cyclic AMP); and, eventually, a change is triggered in the cell's function (for example, the cell divides).

## IS 1080 AN ENDOCRINE DISRUPTOR?

The Agency report (Appendices to the Evaluation and Review Report B16.3.2 p 335) denies that 1080 is an endocrine disrupting chemical because the “ ability to bind to endocrine receptors” has not been demonstrated. However in my submission (No 9301) I provided an important 1977 reference entitled “ *Insulin-like effects of fluoroacetate on lipolysis and lipogenesis in adipose tissue.*” (Taylor et al, 1977) – The beginning of the abstract reads –

“ *Hormone –stimulated lipolysis\* in adipose tissue (\*break-down of fat to use as an energy source) was inhibited by fluoroacetate and there was a concomitant decrease in both the basal and hormone-stimulated cyclic AMP levels. Adenylate cyclase activity in membrane preparations was inhibited by fluoroacetate.*”

(hormone-sensitive **adenylate (or adenylyl) cyclase** is a model system for the study of receptor-mediated signal transduction. It is comprised of three types of components:1) receptors for hormones that regulate the synthesis of a second messenger called cyclic AMP ( cAMP), 2) regulatory GTP-binding proteins (G-proteins), and 3) the family of enzymes called adenylyl cyclases. Concentrations of cAMP are altered by at

least 35 different stimulatory or inhibitory hormones and neurotransmitters. (Krupinski 1991); the importance of this ubiquitous signal transduction system and 1080's shown ability to interact with it for endocrine disruption is made clear below.)

Overhead of the adenylate cyclase system shown with a comment on how adenylate cyclase is a classic G-protein coupled receptor involved in endocrine receptors responsive to a number of hormonal signals for which Alfred Gilman & Martin Rodwell received the Nobel Prize in Medicine in 1994. Hence Taylor et al., 1977 paper shows that fluoroacetate binds and blocks an endocrine receptor system!

(The ERMA scientific advisors in their section on endocrine disruption [ B16.3.2 page 335] have ignored and failed to mention recent advances in our understanding of endocrine disruption which can negatively affect the health of an organism and their progeny which involve many more mechanisms and target organs than those in the Advisory report, with the essence of an endocrine disrupting chemical in being able to interfere with the ' chemical communication' that co-ordinates signaling within an organism and between other organisms ( Fox 2005; Fisher 2004; Tabb & Blumberg 2006; Henly & Korach 2006;Guillette Jr 2006; Grun & Blumberg 2006; Whitehead & Rice 2006;Edwards & Peterson Myers 2007). They limit themselves to the narrow and old mechanistic thinking that an endocrine disrupting chemical involves interference via an oestrogen and/or androgen steroid hormone receptor-mediated mechanism.

The Agency report's use of studies done by Tremblay, Fischer & Leusch (2002) and Tremblay et al (2005) which showed that 1080 and fluorocitrate failed to bind to mammalian oestrogen or androgen receptors, and on the basis of these studies conclude that "1080 is not operating via endocrine disruption". The results of such studies which are based on "**classical**" pathways whereby hormones (such as oestrogen) mediate many of their physiological actions by binding to specific receptors in the nucleus of a cell, which then activate the transcription of target genes, are irrelevant in light of many studies done in the past decade that show that there are "**non-classical**" pathways for hormone effects independent of such oestrogen and androgen receptors (Vasudevan & Pfaff 2006; Das et al, 1997; Moriaty, Kim & Bender, 2006; Syed et al, 2005)

But to understand as to why I quoted the Taylor paper above, with the emphasized words dealing with an intra-cellular messenger called **cyclic AMP**, an enzyme called **adenylate cyclase** and the diagram showing the transmembrane **G-protein coupled receptor** system associated with these 2 factors, and how fluoroacetate interacted with them, is because of vital new research which has shown that some of oestrogen's effects may be mediated by a non-classical pathway in which oestrogen binds to non-oestrogen membrane proteins such as a G-protein coupled receptor called GPR-30 ( Revankar et al, 2005; Hasbi, O'Dowd & George, 2005). Furthermore, Dubey, Tofovic & Jackson (2004) note that

*" accumulating data provide convincing evidence that some metabolites of estradiol, the major estrogen secreted by human ovaries, are biologically active and mediate multiple effects on the cardiovascular and renal systems that are largely independent of estrogen receptors"*

Recently Steegborn et al (2005) has shown that the type of oestrogen (=estrogen) metabolites called catechol oestrogens can elicit physiological responses by binding to cellular targets, one such ligand-binding site involved binding and inhibiting transmembrane adenylyl cyclases ( remember 1080 was shown by Taylor et al, to be an adenylate cyclase inhibitor).

Possible 1080 endocrine disrupting action on the production of ovarian oestrogen and progesterone production in addition to androgen synthesis in mammalian testes, needs to be evaluated as the expression and activity of the key mitochondrial transmembrane protein called steroidogenic acute regulatory protein (StAR) is controlled by G-protein regulated receptors which on binding to the pituitary hormones called FSH and LH, leads to adenylyl cyclase stimulated increased cAMP levels to produce the aforementioned hormones ( Jamnongit & Hammes, 2006). Also regulation of sperm function has been found to be by adenylyl cyclase signaling pathways (Fraser et al, 2005).

FSH & LH are also key players in the regulation of the process called folliculogenesis, that is, the of growth and functional maturation undergone by ovarian follicles, from the time they leave the pool of primordial (quiescent) follicles until ovulation, at which point they release a fertilizable oocyte or egg. Both FSH and LH operate mainly through G protein-coupled transmembrane receptors, transducing their signal by activation of the enzyme adenylyl cyclase and production of second-messenger cAMP. 1080 induced adenylyl cyclase inhibition would be a major endocrine-disrupting action. So it is paramount to check if sublethal 1080 doses influence mammalian oocyte(egg) development as G-protein regulated adenylyl cyclase activity and intra-cellular cAMP levels are important in oocyte/egg maturation.( Richards 1980, Richards 1988; Jamnongjit & Hammes 2005).

The oestrogen G-protein coupled receptor 30 has recently been identified in the rat central nervous system, showing a high level of presence in the hypothalamus and pituitary, key areas of the brain involved with hormonal regulation ( Brailoiu et al, 2007).Such a G-protein coupled receptor in the brain may be the endocrine-interfering mechanism for fluoroacetate leading to an attenuated release of luteinizing hormone-releasing hormone from the rat hypothalamus found by Wu, McArthur & Harms (1991).

G-proteins control many diverse pathways of transmembrane signaling (Freissmuth, Casey & Gilman, 1989; Meij, 1996), and if 1080 does interfere with them, then there will be a multitude of problems, including interference with thyroid function which has profound implications in the early stages of human or mammalian development. Thyroid stimulating hormone receptors belongs to the same class of G-protein receptor coupled receptors as luteinizing hormone receptor and follicle-stimulating hormone receptor (Laugwitz et al 1996; Puett et al, 2007).

Endocrine disruption or interference in signaling communication may not just occur with an organism but between different organisms, for example, haloacetate analogues, including monofluoroacetate have been found to interfere with insect communication systems and moth pheromone communication ( Pesenti & Viani 2004; Camps et al, 1990; ) and the mechanism it probably via an Orphan G-protein –coupled receptor ( Dong-Soon , 2002). **So the G-protein coupled receptors are the most likely mediators for 1080 endocrine disruption.** If that is the case then given the ubiquitous presence in signaling pathways in cells throughout the animal and plant kingdom, 1080 endocrine disrupting potential will be much more widespread than acknowledged and altered gene expression and functional consequences would be most dangerous in the early developmental periods ( see below for further discussion on this most worrying aspect and hazard).

So I have provided scientific evidence which the Agency report could not or did not find when they noted in their criticism of Weaver (2003) that to show 1080 may have endocrine disrupting activity, it is necessary to show how 1080 causes endocrine/reproductive related effects that “ are mediated by interference in the endocrine hormones and receptors”.[Appendix B16.3.2 page 335]

Although the technology exists to clarify the transmembrane proteins that bind to fluoroacetate and possible endocrine-disrupting interactions, little research has been done using modern means. It's about time the manufacturers and users of 1080 spent some of their money on such fundamental mechanistic research, having had decades of ignoring this issue.

(Warning bells regarding the significant mechanistic toxicological effects of 1080 can be deduced on the basis of Kun's 1982 and Taylor et al, 1977 studies. Kun bemoaned the lack of basic research in his 1982 paper and little has been done since to clarify how fluorocitrate interferes with such membrane processes and protein macromolecules which will interfere with many aspects of cell signaling pathways with a multitude of effects. A multitude of new techniques in molecular biology exist now to explore this today.)

## MITOCHONDRIAL DNA MUTATIONS

I believe 1080 is especially an endocrine-disrupting chemical in the earliest stages of development and that it can also lead to *mitochondrial (mt) DNA mutations* through the process of “*reductive stress*” (de Grey 2000, de Grey 2002) and impaired mitochondrial DNA maintenance (Chen et al., 2005), which is beyond the scope of this talk.

(*Mitochondrial DNA* is DNA or genetic material separate from that on chromosomes called nuclear DNA and is contained within the energy-producing subcellular structures called mitochondria.

Mitochondrial DNA mutations are specific changes in the normal mt DNA associated with disease.

Human mitochondrial DNA mutations can cause human diseases, once thought to be the domain of purely academic interest due to their rarity, epidemiological studies in recent years have confirmed that mitochondrial diseases are among the most common genetic disorders and a “major burden” on society (Dimauro & Davidzon, 2005) with estimates of individuals affected in a British population being about 1 in 3,500 people thought to either have mt DNA disease or are at risk of developing it (Schaefer et al, 2004). More recently considerable interest exists in the possibility that mt DNA variants and mitochondrial dysfunction predisposes to common diseases such as diabetes, Alzheimer disease, Parkinson’s disease and certain cancers. (reviewed in Taylor & Turnbull, 2005).

All mtDNA at fertilization comes from the oocyte or female egg. Therefore, inherited mtDNA mutations are transmitted from the mother to all offspring, male and female alike. The higher the level of mutant mtDNA in the mother, the higher is the frequency of clinically affected offspring and the more severely the children tend to be affected.

Each cell in the body contains different mtDNA populations. Due to presence of multiple mitochondria in cells, each cell contains several mtDNA copies. This produces tissue variation so that a mutation in mtDNA vs normal mtDNA can vary widely among tissues in an individual.

There is thus a threshold effect. The percent of mutant mtDNAs must be above a certain threshold to produce clinical disease. This threshold varies from tissue to tissue because the percent of mutant mtDNAs needed to cause cell dysfunction varies according to the oxidative requirements of the tissue, affecting particularly organs with a high energy needs such as brain and muscle. (Rossignol et al, 2003)

### ***Can sublethal exposures to 1080 cause mitochondrial DNA (mt DNA) mutations which may lead to disease?***

The recent finding that mitochondrial aconitase, the enzyme to which the 1080 metabolite fluorocitrate may form an inhibitor-complex, is essential to mt DNA maintenance, independent of its catalytic activity, and that aconitase functions to stabilize mt DNA, perhaps by reversibly remodeling of protein-DNA complexes called nucleoids to directly influence mitochondrial gene expression in response to changing cellular metabolism (Shadel, 2005; Chen *et al.*, 2005). Sublethal 1080 doses may interfere with mitochondrial metabolism to the extent that mitochondrial DNA integrity is compromised and thus more vulnerable to mutations and limited in their inability to repair them. I note the important Chen paper, which I provided in my written submission **was omitted from the Agency report reference list**- perhaps the title would arouse concern in anyone with scientific expertise, as a possible mechanism for mitochondrial DNA mutation can be inferred from it.

Inactivation of aconitase by 1080 may lead to a state called “*reductive stress*” where normal electron flow to oxygen is blocked in mitochondria, leading to an accumulation of reduced metabolites such as NADH. This condition can cause increased reactive oxygen species production through a process called autoxidation of the reduced metabolites. Free radicals are reactive substances that readily form harmful

compounds with other molecules, that can damage the mitochondria over time, causing damage that can not be reversed ( de Grey 2000, de Grey 2002, Stadtman ER, 1988). Mitochondrial DNA, unlike nuclear DNA, has very limited repair abilities and almost no protective capacity to shield the mitochondria from free radical damage. One of the protective mechanisms involves the previously mentioned glutathione system which fluorocitrate can bind and in the young deplete rapidly, further limiting availability to protect the mitochondria DNA.

Also Yan *et al.*, (1997) paper indicated that fluoroacetate's ultimate effect is similar to that caused by that of experimentally induced oxidative stress. They showed that when flies were exposed to different concentrations of fluoroacetate in their drinking water, the aconitase inhibitor caused a dose-dependent decrease in life-span. The maximum life-span of non-treated houseflies under experimental conditions was 47 days, and this was shortened to 38, 30 & 17 days by addition of 1, 50 and 100 ppb ( $\mu\text{M}$ ) fluoroacetate to the drinking water. This decrease in life-span was also induced when the flies were exposed to hyperoxia (100% oxygen) to cause oxidative damage to the aconitase.

What effect on human aging does chronic sublethal doses of 1080 cause? Recent research involving the creation of a mt DNA mutator mice, has provided the first evidence that accelerating the mt DNA mutation rate can result in premature aging (Trifunovic 2006). Chronic sublethal exposures may lead to accumulated mt DNA mutations and eventual disease.

Perhaps the adult-workers that handle 1080 over chronic periods should be tested not for 1080 levels but somatic ( that is body cells other than the male sperm and female egg or so called germ-line cells) mitochondrial DNA mutation rates which may be a truer reflection of any sub-lethal toxic effects which may explain some of the findings of the Parkin et al, 1977 case of chronic 1080 poisoning of a rabbit, which the Agency report wrongly discards.

#### ***Does such oxidative stress cause cancer?***

Bolt et al ( 2004) note that oxidative stress in causing cancer, is an important mechanism triggered by reactive oxygen species ( ROS=free radicals) and that ROS-mediated processes of carcinogenesis at low doses needs to be considered by regulatory bodies in Europe. As mentioned above, 1080 can cause reductive stress associated with ROS formation. Sublethal doses of 1080 may lead to alterations in the reduction/oxidation status of cells so that cellular growth and differentiation is altered, for instance a more reducing environment or "reductive stress" state has been linked with the proliferation of some tumour(cancer) cells and with impaired ability of a sublethal 1080 exposed mitochondrion to produce ATP or energy, may lead to altered NAD<sup>+</sup>/NADP ratios which can disrupt signaling pathways (Giles 2006).

With respect to the Agency report (Appendices to the Evaluation & Review Report B11.4 page 305) on the basis of the negative in-vitro studies ( Eason et al, 1999) and a negative in-vivo study provided "sufficient information to conclude that 1080 does not trigger mutagenicity". The Agency report also states that there are no long-term (life-time) toxicity or carcinogenicity bioassays using 1080 and hence " on the basis of the negative mutagenicity data for 1080, the Agency, I believe, falsely concludes that " there is no reason to suspect that 1080 may be carcinogenic." ( pg 306).

Regulatory bodies should be made aware that there is growing evidence that the paradigm "carcinogen=mutagen" is now believed to be too simplistic and in their position paper, Trosko & Upham ( 2005) state that current genotoxicity studies and the rodent bioassay to predict human cancer risks are no longer useful or relevant given developments in the modern understanding of carcinogenesis. In an aptly titled paper *-The Emperor wears no clothes in the field of carcinogen risk assessment: ignored concepts in cancer risk assessment.* Trosko & Upham state that routine human carcinogenic hazard and risk assessments ignore " 'epigenetic' effects of carcinogens and role of cell communication systems in epigenetically altering gene expression that leads to an imbalance of cell proliferation, differentiation and apoptosis {or programmed cell death} in a tissue that can contribute to the cancer process." They draw attention to the fact " that the current paradigm and policy to test toxic chemicals is often misleading and

incorrect....**oxidative stress**, in spite of the DNA damaging data, most probably contributes to cancer at the epigenetic level.”

***What does ‘epigenetic’ mean?***

In the not too distant past, gene expression, was thought to be determined solely by DNA-base sequences, now we know it also depends upon **epigenetic phenomena**, defined as gene-regulating effects that do not involve a change in the DNA-base sequence but change DNA function and can persist through one or more generations. (Pennisi, 2001). Although the cells of the body contain essentially the same DNA sequence, they express very different sets of genes, epigenetics plays a central role in how the patterns of gene expression, the switching ‘on’ and ‘off’ of genes may be altered by exposure to environmental factors, including pesticides such as 1080.

Think of genes as messages or words which bring about actions or responses in a cell, epigenetics deals with the timing of expression and meaning of those messages or words which can be different without changing the actual letters of the word, by mechanisms such as the way the word or messages are delivered ( e.g sung, screamed, whispered, e-mailed, phoned, recorded and played later, associated with hand gestures, a hand holding something like a flag etc ) and where the recipient cell can remember and “read between the lines” and know what the message means and respond accordingly. The message may be altered by timing, and some may be unforgettable and irreversibly alter the way a cell/tissue/organ/organism develops and behaves for the rest of its life. If 1080 exposure occurs at a early stage of development but not sufficient to kill, then the message may mean and bring responses that are totally different than if given at another time – say adolescence or adulthood. The early memory of such an exposure may not limit itself to just one’s life-time, that early word may lead to altered gene expression with disease susceptibility or later disease manifestation, and this phenomenon can be remembered and passed onto future generations with the similar meaning and adverse response persisting.

Unfortunately epigenetic mechanisms of endocrine disruption involving altered gene expression, mediated by inappropriate activation or deactivation of receptors that act have now been identified for environmental chemical contaminant exposures which can lead to disease such as infertility, cancer, obesity, neurodegenerative diseases, diabetes etc (Edwards & Peterson Myers, 2007). I believe that 1080 would belong to this category of epigenetic toxicants for early human and animal life and manufactures & users should be made to prove that this is not the case. .

**THE MOST DANGEROUS AGE IN LIFE TO BE EXPOSED TO 1080 AND IT IS COMPLETELY OMITTED IN THE AGENCY REPORT.**

No where in the Agency report is my most important submission comment evident, namely:

***“ Sublethal dose effects of 1080 at the earliest stages in human life needs to be researched as a mitochondrial –based model for fetal origin of adult disease ( Lee et al 2005) shows that perturbed initial condition of mitochondrial function can lead to reduced insulin sensitivity leading to metabolic syndrome, which is associated with diabetes, hypertension, dyslipidaemia, and obesity. A recent review ( Knudsen & Green, 2004), supports the provocative hypothesis that environmental programming of mitochondrial status during early life may be linked to diseases that manifest only in adulthood.”***

(For instance a pregnant woman who has consumed watercress and contaminated eel on a single occasion with her whanau, may receive minute sublethal 1080 doses that do not harm her as an adult, but may be very dangerous for her baby during critical periods of development inducing very subtle mitochondrial dysfunction by transmembrane altered intracellular signaling that involve cellular “stress” that may result in

subtle but permanent epigenetic changes – with altered reprogramming of gene expression, also known as metabolic imprinting (Waterland & Garzo 1999), which may lead to disease years later and possibly be passed onto future generations. They may also lead to mtDNA mutations through ROS as mentioned above.

New Zealand has one of the foremost experts in this area – Prof Peter Gluckman. We should be studying this to protect future generations of NZ children, especially at-risk rural children. Current generations may already be suffering disease burden from previous parent or grand-parent exposures. Models for studying this serious possible hazard exists today. It's about time 1080 research entered the post-genomic age. It is highly possible that less than 3.5 parts per billion of 1080 exposure could adversely affect human embryo development.

(Professor C. Vyvyan Howard, who is on the British government's advisory committee on pesticides, commented last year that low levels of chemicals from pesticides could affect the development of babies before they are born and increase their likelihood of developing cancer later in life and that his research (Newby & Howard 2006) indicated that the dangers of pesticides had been underestimated and said **“ We're talking about chemicals which could possibly cause cancer in children at parts per billion and parts per trillion levels, rather than parts per million or thousands.”** (cited and retrieved on 24/03/06 <http://www.guardian.co.uk/food/Story/0,,1735687,00.html> )

*The 'playground- primary school' shortcomings of toxicological end-points found in Eason et al developmental and subchronic toxicity studies.*

Eason (1999), Eason & Turck 's (2002) simplistic research methodology cannot be used to predict all target organ 1080 hazard risks. And their lack of a mechanistic explanation for their observations, for example skeletal malformations, may be helped by the findings of other developmental organ system studies which they did not address- for instance Saitu (1984) found fluoroacetate caused severe structural changes in the cells responsible for dental enamel formation called ameloblasts, which are very sensitive to any perturbations in their environment and some recent research (Kubota et al, 2005) suggests that this is due to fluoride-mediated stress response gene activation that leads to abnormal protein folding.

To clarify the importance of this for the non-scientist panel members, an analogy may be useful; if the organ systems of a study animal were equated with computer “hard-ware” then the sub-lethal exposures to 1080 in developmental fetal stages or chronic sub-lethal exposure over 90 days in mature animals would not tell you if any damage or ‘epigenetic’ reprogramming had been done to the “soft-ware” which may lead to systems failures at a much later date. The analogy eventually breaks down – because alterations in gene-expression (soft-ware reprogramming) following sub-lethal 1080 exposure that cause no immediate overt visible damage, may lead to impaired mitochondrial function which eventually can physically change the hard-ware manifesting as cancer or the diseases listed above – diabetes, hypertension, abnormal cholesterol/lipids, obesity, or possible neurodegenerative disease and fertility problems which have all been associated with mitochondrial dysfunction- all areas/functions not able to be detected by. Eason 1999 and Eason and Turck 2002 – their end-points were the physically, visibly damaged “hardware”- such as skeletal deformities, testicular and heart muscle abnormalities. However disease-resulting from long-term changes in many other parts of the body affected by any post-1080 altered “soft-ware” cannot be detected by their method of research. – that requires using 21<sup>st</sup> century technology involving toxico-genomics or 2-generation studies or even better seeing if the altered soft-ware programming leads to disease(s) passed on to at least 4 generations as some recent evidence shows this may be possible– [see below female reproductive section]; hence the absolute horror I have for the Agency report advising that such studies are not necessary. If the software programming is altered in the earliest stages of human development or other living creatures during critical periods of their early development, then such inheritable disease or disease susceptible states may be irreversible and this alone demands stopping 1080 use until the research has been done by independent sources to allay this possible mechanism of immeasurable negative health consequence.

Mitochondrial DNA mutations or epigenetic mechanisms may lead to a plethora of diseases and possible alterations in mitochondrial gene expression with functional consequence that may lead to metabolic syndrome, obesity, heart disease, diabetes to which Maori and Polynesians are over-represented in morbidity (Gentles et al, 2007) and mortality figures in this country. The role of 1080 or other pesticides inducing possible mitochondrial DNA mutations or epigenetic changes in gene expression in these populations is yet to be researched. Yet some rural populations in New Zealand may be suffering from 1080 exposures from decades ago, hence there is a cultural and ethical obligation to exclude this possibility – or perhaps, depending on the final panel decision and recommendation, I will inform them (especially rural Maori ) of this potential serious risk which is yet to be excluded; moreover I may collaborate with a specially set up group to fund independent researchers to do some of the key studies necessary if the appropriate recommendations and mandate for the research to be done prior to any further aerial 1080 drops are not made mandatory. )

***Possible role of sublethal doses being a developmental neuro-toxic substance.***

Recent research looking at the developmental neurotoxicity of the insecticide chlorpyrifos maybe pertinent to 1080 and similar types of studies could be done using such methodology for the latter. Chlorpyrifos in low non-toxic doses during various vulnerable periods of prenatal and early neonatal periods has been found to alter cAMP levels ( critical for brain development) and target the adenylate cyclase (AC) pathway at the level of AC expression and G-protein function, that results in long-term alterations in neural cell signaling pathways with resultant altered gene expression (an epigenetic effect) that leads to latent behavioural & cognitive deficits that manifest in adolescence and that persist into adulthood ( Meyer et al, 2004;Levin et al, 2002); also of note was the effect show by chlorpyrifos exposure during early critical periods involved developmental toxicity with delayed -onset effects of altered adenylate cyclase signaling in liver and heart cells ( Meyer et al, 2004). Given the above discussion of 1080 being able to target adenylate cyclase and G-coupled proteins and the fact that cholinergic function with the brain neurotransmitter acetylcholine can be inhibited by 1080, then developmental toxicity studies looking for interference in cell signaling pathways/cascades should be studied to check for any sublethal dose latent effects on the brain and any long term behavioural adverse effects as well as long-term liver and heart pathology

Now I believe that any child who has suffered any 1080 induced adverse health effects due to mitochondrial dysfunction would be hard to diagnose as they may present with any symptom and any tissue, any organ system may be affected, although the central nervous system, heart and skeletal muscle are more prone, and diagnostic testing is beyond the capability of most GPs and many may not consider 1080 as a likely cause..

Shanske, Shanske & DiMauro (2001) note:In the past 13 years, a new chapter of human genetics, "mitochondrial genetics", has opened up and is becoming increasingly important in differential diagnosis. Although the clinical manifestations of disorders related to mitochondrial DNA (mtDNA) are extremely variable, recent advances in genetic testing aid in the identification of patients. Muscle morphology can give important clues for diagnosis, but histological features alone cannot define a specific disorder.

Biochemical analysis may reveal a single enzyme defect, or when multiple activities are affected, suggest an mtDNA mutation. However, definitive diagnosis often requires DNA analysis and documentation of a specific mtDNA abnormality. Disorders associated with mtDNA mutations are associated with a wide variety of syndromes, and owing to the properties and characteristics of mtDNA, these are often transmitted by maternal inheritance. Although therapy for mitochondrial diseases is limited, identification of the molecular defect is important for genetic counseling.

**LACK OF REPRODUCTIVE STUDIES & MULTIGENERATION STUDIES, ESPECIALLY EFFECTS OF 1080 ON THE FEMALE FOLLICULAR AND OOCYTE (EGG) DEVELOPMENT IS UNACCEPTABLE.**

Another area of neglect and great concern is that 1080 may interfere with the reproductive systems, especially of females during periods of oocyte (egg) and embryonic follicular development (Kane & Buckley 1977, Wycherley 2005). This involves germ-cell lines and the fact the mitochondrial DNA is maternally transmitted.

(The father's sperm loses its mitochondrial DNA at fertilization, all mitochondrial DNA is derived from the oocyte/egg, which means that a mother carrying a mitochondrial DNA mutation or altered epigenetic programming, will transmit it to all her children, females and males, but only her daughters will transmit it to the next generation.)

1080 could negatively affect multiple future generations by epigenetic mechanisms. There is recent evidence (Anway & Skinner 2006, Chang et al, 2006) for male germ line trans-generational epigenetic effects for ***increased disease for four subsequent generations*** after one short pesticide exposure during reproductive organ development. (implying the possibility of a great-great grandchild may be affected with fertility problems, cancer, renal disease, abnormal immune function etc by some chemical they were never exposed to nor their parents, nor their parents. The research techniques now exist to study any epigenetic effects on the female reproductive germline cells as well as the male one.) This has serious implications for not only humans, but mammals, birds and fish if exposed at a critical period of development to 1080. Yet the Agency report deems this as not being an important factor in their risk assessment!

In conclusion an overhead with a diagrammatic healthy functioning ATP-energy producing mitochondrion with normal membrane machinery and minimal free radical production was shown next to an accompanying pictorial representation of a putative 1080 damaged mitochondrion with impaired ATP( energy) production and increased free-radical damage with distorted inner and outer membrane shape to illustrate that 1080 is not just a Krebs cycle ***metabolic inhibitor*** but that it will have profound effects on all mitochondrial functions involving many diverse biological roles ( aging, redox sensing, chronic disease susceptibility, etc) other than just metabolism – 1080 at sublethal doses may cause subtle and hidden ***mitochondrial dysfunction***.

If you interfere with mitochondrial function then you interfere with some of the most intimate functions of a living organism as Nick Lane explores in his popular science book ***Power, Sex, Suicide: Mitochondria and the Meaning of Life*** where he takes the mito-centric perspective and its critical role in not only energy production ( power), but in sexual reproduction (sex) and the process of programmed cell death or apoptosis (suicide) reviewed by John Alcolado ( 2005)

My work is supported by the recent International Conference on Fetal Programming and Developmental Toxicity involving over 200 of the world's leading environmental scientists and experts whose Faroes statement 24/05/07 on Human health effects of developmental exposure to environmental toxicants notes “ even subtle effects caused by chemical exposures during early development may lead to important

functional deficits and increased risk of disease later in life.” The ERMA Advisory report needs to move away from the “dose makes the poison” approach and its false reassurances and many scientifically poor recommendations and engage the new paradigm that toxicological assessments must now give priority to timing of exposures during critical periods of development, “ the timing makes the poison” with the youngest human being’s in the womb is the most vulnerable to possible adverse episodic, extremely low sublethal 1080 exposure. They must recognize that “ the physiological mechanisms involved in the development of energy and nutrient metabolism are also highly vulnerable to toxic effects of environmental chemicals” – 1080 interferes with energy and nutrient metabolism.

<http://www.pptox.dk/Consensus/tabid/72/Default.aspx>

This demands urgent action by all regulatory bodies, including the current panel, to take note of some of the expert panels recommendations :

- 1) Environmental chemical exposure assessment should emphasize the time period of early development
- 2) Toxicological tests and risk assessment of environmental chemicals needs to take into account the susceptibility of early development and long-term applications of adverse programming effects
- 3) The accumulated research evidence suggests that prevention efforts against toxic exposures to environmental chemicals should focus on protecting the fetus and small child as highly vulnerable populations. .... Such prevention should not await detailed evidence on individual hazards to be produced, because delays in decision-making would lead to propagation of toxic exposures and their long-term consequences. Current procedures therefore need to be revised to address the need to protect the most vulnerable life stages through greater use of the precautionary approaches to exposure reduction.

The Advisory report misleads and selectively uses science to hide behind and defend an indefensible position in light of the most up-to-date information with respect to the issue of mechanism of toxicity, endocrine-disruption, developmental, reproductive and other health related risks. If only one NZ mother has suffered and her child has been an innocent victim of bad health, because of false safety reassurances and indifference, then all the monetary savings for any pest control in the world, simply can not do that child and mother any moral justice and their health can never be bought back and their future progeny must suffer as well. And I know one such mother who believes that 1080 has caused her children and family such grief.....

***“An observant parent’s evidence may be disproved but should never be ignored.”***

( Anonymous- Lancet 1951;1:688)

***“ We simplify Nature’s complexity at our peril.”***

(Robert Chapin)

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