Report on *'Fluoride and Cardiovascular Disease: Implications for Public Health in Ireland and other Fluoridated Communities by Declan Waugh'*

This article is not peer-reviewed which is a major concern and reflects the poor scientific quality of this manuscript. Several statements lack scientific justification and some of the reported studies are wrongly interpreted. Several references are cited multiple times and some of the references don't support he statement made in this article. It is also important to note that most toxicological/pathological studies cited in this article have used very high doses of fluoride and are hence not appropriate to correlate the findings observed in these studies with any adverse effects of fluorinated drinking water consumption on cardiovascular diseases. My details comments on each of these studies are included below:

Page no	Comment made by the author	Peer review of the comment
	Refs 66 and 65 are same E.A. Garcia-Montalvo, H. Reyes-Perez, L.M. Del Razo, Fluoride exposure impairs glucose tolerance via decreased insulin expression and oxidative stress, Ref 56, 68, 36, 129, 146 are same O. Barbier et al. Molecular mechanisms of fluoride toxicity, Chemico-Biological Interactions *188 (2010) 319–333* Ref 60 and 90 are same USA National Research Council, Fluoride in Drinking Water: A Scientific Review of EPA's Standards, Committee on Fluoride in Drinking Water, (2006), Page 263 Ref 114, 141 are same Ercan Varol ÅSimge Varol, Effect of fluoride toxicity on cardiovascular systems: role of oxidative stress, Arch Toxicol (2012) 86:1627	Several references are cited multiple times with different reference numbers. It is not appropriate to cite review articles for certain scientific statements. In few instances (detailed below) the references cited don't in any way support the statement/s made.
Page 7-8	The ability of fluoride to impair the bodies immune and respiratory system (Ref no 36-38) The inflammatory effect of fluoride exposure has also been demonstrated in human lung epithelial cells.[38]	It is important to note that in the references cited: the rats were fed on 10, 50 and 100 mg/L NaF (i.e., 10, 50 and 100 PPM) in drinking water (Ref no 36). The pathological effects were observed only in 50 and 100 mg/L NaF treated groups and not in groups receiving lower doses. Moreover the control group of rats receiving ~ 1 PPM of F in water didn't show any of the pathological abnormalities. It is necessary to note that the NaF dose added was on top of F already present in the drinking water (1 mg/L ~ 1 PPM). The above doses correspond to 10, 50 and 100 PPM, which is a dose 10-14 times above the concentration in drinking water (0.7-0.9 PPM). Only lung abnormalities were reported in this study and none of the direct cardiovascular parameters were evaluated in this study. Hence

		it not appropriate to link the findings from this study with cardiovascular concerns. Ref no 38: is a cell based assay: in this study NaF was evaluated at 2.5, to 5 mM concentration. This concentration corresponds to 102 to 205 PPM, which is again much higher concentration. It is essential to note that in a cell based assay the high concentration of fluoride indicate is directly exposed to lung epithelial cell cultures under static conditions, such high concentration of fluoride will never be achieved invivo by consumption of drinking water fluorinated at 0.7-0.9 PPM.
Page 10	Fluoride is a risk factor in both the development of obesity and diabetes. Ref no 55	Ref 55 is a review article and having read this review, I didn't come across fluoride being indicated as a risk factor for development of obesity and diabetes. Moreover none of the cross references cited in this review support and provide scientific evidence for fluoride being a risk factor in development of obesity and/or diabetes.
Page 10	Ref 57 cited multiple times (Ref 56, 68, 36, 129, 146 are same) It is now known that biologically relevant doses of fluoride results in impairment of glucose tolerance or increased blood glucose and decreased insulin synthesis. Ref no 56	Ref 57 is again a review article: None of the studies reported in this review have studied the effects of NaF or Fluoride alone in biologically relevant doses; Below I have included the details on the fluoride concentrations used in each of the cross references mentioned in this review article. Table 1 in this review gives a summary of most in vitro and in vivo studies to date looking at antioxidant enzymes by NaF **In vitro (animals cells)** Mouse pancreatic beta-cells at 1.35 and 2.5mM for 12 h: i.e., 55.35 and 102.5 PPM NaF concentration Primary rat hippocampal neurons at 20, 40, and 80 mg/l exposed for 24 h: i.e., 20, 40 and 80 PPM NaF concentration Murine hepatocytes at 100mM for 1 h i.e., 4100 PPM NaF concentration **In vitro (human cells)** Hepatocellular carcinoma (HepG2) cells at 3mM for 6 and 24 h; i.e., 123 PPM NaF concentration Neuroblastoma (SH-SY5Y) cells exposed at

		0.05ï 5mM for 24 h; i.e., 2- 205 PPM NaF concentration Human hair follicles exposed at 1.0, and 10mM for 5 days; i.e., 41 and 410 PPM NaF concentration **In vivo (animals)** Male albino guinea pigs exposed at 250mg NaF/kg subcutaneously and sacrificed 8 h Later i.e., 250 PPM NaF concentration Male Wistar rats exposed at 5mg/kg body mass/day, orally for 8 weeks i.e., 5 PPM NaF concentration. Male Swiss mice exposed at 50 mg/l in drinking water for 10 weeks. i.e., 50 PPM NaF concentration. Albino rats exposed at 100 mg/l in drinking water for 4 months. i.e., 100 PPM NaF concentration. Male albino Wistar rats exposed at 1, 10, 50 and 100 mg/l in drinking water for 12 weeks. i.e., 1, 10, 50 and 100 PPM NaF concentration. Interestingly in this study pathological findings were evident only in 50 and 100 PPM NaF group and not in the lower dose (1, 10 PPM) group. Second generation of Male Albino adult Wistar rats exposed at 10, 50, and 100 mg/l in drinking water for 180 days. i.e., 10, 50 and 100 PPM NaF concentration. Chicks exposed by diet to 100, 250, or 400mg F/kg for 50 days. i.e., 100, 250 and 400 PPM F concentration. Male albino rats exposed at 10.3mg NaF/kg body weight/day, orally for 5 weeks. i.e., 10.3 PPM NaF concentration. Pig exposed to food supplemented with 250mg F/kg for 50 days. i.e., 250 PPM NaF concentration. Male rats exposed at 20 mg/kg/day for 29 days by oral gavage. i.e., 20 PPM NaF concentration. Male Wistar rats exposed at 50 and 100 mg/l in drinking water during 4 months. i.e., 50 and 100 PPM NaF concentration.

		Male and female Wistar rats exposed at 50,100, and 150 mg/l in drinking water during 3 months. i.e., 50, 100 and 150 PPM NaF concentration. Barrows exposed at 250 and 400 mg/kg (from NaF) in their diets for 50 days. i.e., 250 and 400 PPM NaF concentration. Male Swiss mice exposed at 5mg/kg body mass/day, orally for 8 weeks. i.e., 5 PPM NaF concentration. Female rats exposed at 100 mg/l in drinking water for 60 days. i.e., 100 PPM NaF concentration. Swiss albino male mice exposed at 50 mg/l in drinking water for 3 weeks. i.e., 50 PPM NaF concentration. Male albino rats exposed at 10, 50 and 100 mg/l in drinking water for 10 weeks. i.e., 10, 50 and 100 PPM NaF concentration. Female Albino mice exposed 5mg/kg body weight/day, orally for 30 days. i.e., 5 PPM NaF concentration. Male Balb/c mice exposed at 200 mg/l, in drinking water for 7 days. i.e., 200 PPM NaF concentration. Female Wistar rats exposed at 150 mg/l in drinking water for 28 days. i.e., 150 PPM NaF concentration. Wistar albino pups placentally and lactationally exposed from mother rats at 50,and 150 mg/l in drinking water. i.e., 50 and 150 PPM NaF concentration. **In vivo (human)** Residents from China-endemic area (mean urine concentration of 2mg F/l). i.e., 2 PPM NaF concentration in urine. Children with skeletal fluorosis from Indian-endemic area (mean water concentration of 5.53mg F/l). i.e., 5.53 PPM NaF concentration.
Page 10		Studies evaluating effect of NaF on gene expression in reference no 56 (review article): **In vitro (animals cells)** Primary rat hippocampal neurons at 40, and 80 mg/l for 24 h. i.e., 40 and 80 PPM NaF

		concentration. Porcine enamel organ cells and ameloblast-derived cell line (LS8 cells) at 2mM NaF for 48 h. i.e., 82 PPM NaF concentration. Mouse pancreatic beta-cells at 1.35, and 2.5mM for 12 h. i.e., 55.35 and 102.5 PPM NaF concentration. Primary rat hippocampal neurons at 40, and 80 mg/l, for 24 h. i.e., 40 and 80 PPM NaF concentration. Osteoblasts of Sprague‌ï Dawley rats at 0.05 and 4mM for 72, and 120 h. i.e., 40 and 5 and 164 PPM NaF concentration. Mouse odontoblastos (MO6-G3 cells) at 1mM for 5days. i.e., 41 PPM NaF concentration. **In vitro (human cells)** Ameloblast lineage cells at 10 mM for 24 h. i.e., 410 PPM NaF concentration. Primary gingival epithelial cells at 5, and 50 mM for 24 h. i.e., 205 and 2050 PPM NaF concentration. Pulmonary epithelial (A549) cells at 5mM for 8ï 24 h. i.e., 205 PPM NaF concentration. Hepatocellular carcinoma (HepG2) cells at 3mM at 6 and 24 h. i.e., 123 PPM NaF concentration. Embryonic hepatocytes (L-02 cells) at 40, 80, and 160 mg/l, for 24 h. i.e., 40, 80 and 160 PPM NaF concentration. Neuroblastoma (SH-SY5Y) cells at 40, and 80 mg/l, for 24 h. i.e., 40, and 80 PPM NaF concentration. **In vivo (animals)** Enamel epithelial cells of Wistar rats exposed to 100mg F/l (5.25mM) in drinking water for 8 weeks. i.e., 225 PPM NaF concentration. Liver of pig exposed to food supplemented with 250mgF/kg for 50 days. i.e., 250 PPM NaF concentration. Sperm of Kunming mice exposed to 70, and 150mgNaF/l (3.7, and 7.9mM) in drinking water for 49 days. i.e., 70 and 150 PPM NaF concentration.

		In vivo (humans) Peripheral blood mononuclear cells from Mexican individuals drinking water with levels of 1.9ï 4.02mg F/l. i.e., 1.9-4.02 PPM NaF concentration. Peripheral blood mononuclear cells from individuals living in endemic area in China (mean urine concentration of 2mg F/l). i.e., 2 PPM NaF concentration in urine.
Page 11	Researchers Menoyo et al.63 and Lin et al.64 demonstrated the effect of fluoride on glucose metabolism using in vivo and in vitro experimental models and confirmed that biologically relevant doses of fluoride result in impairment of an oral glucose tolerance test and decreased insulin synthesis.	Ref 63: 5-20 micromol/L (~ 0.21 ï 4.05 PPM) in the extracellular space inhibited insulin secretion by isolated Langerhans islets stimulated with glucose. However this effect was reversible. Considering fluoride absorption kinetics it is unlikely to achieve these concentrations in pancreatic extracellular space with consumption of 0.7-0.9 PPM fluorinated drinking water. Moreover in this study only 20 micromol/L fluoride concentration (~ 4 PPM) was partially inhibitory in the invivo model (perfusion of rat pancreatic tissue) and these effects were reversible. In Ref no 64: 17 mM fluoride was used i.e., 697 PPM NaF
Page 11	It has also been reported by Montalvo et al. (ref 65) that fluoride exposure regulates insulin gene expression in murine beta pancreatic cells, resulting in reduced insulin secretion. Ref 65 and 66 are same	In this study Mice received 45mg/L (~45 PPM), as NaF, via drinking water, and cells were exposed for 12 h to NaF (equivalent to 0, 0.007, 0.045, 0.180, 1.35 or 2.26mM F) at a basal or stimulatory glucose concentration (2.8 or 16.6mM, respectively). Only cell exposed to 1.35 and 2.26mM NaF (~ 22.35 and 99 PPM NaF) had significantly lower insulin and this effect was not observed with lower concentrations of NaF. The effect reported was evident only at higher NaF concentrations (> 22 PPM).
Page 11	Fluoride exposure may contribute to impaired glucose tolerance or increased blood glucose Ref 66 (ref 66 and 65 are same), 67 and 68 (this ref is discussed above)	Ref 67: 5 mM NaF solution (~ 205 PPM NaF). Hence the authors claim on the possibility this being induced by fluoride levels in drinking water is not correct and not scientifically supported.
Page 11	An examination of the fluoride as a risk factor in both diabetes and Obesity in Ireland has previously been examined by	Self-citation. This reference again is not peer-reviewed and may lack scientific credibility as the current manuscript.

	Waugh.[69]	
Page 11	In humans, effects on thyroid function have been documented with fluoride exposures of 0.05-0.13 mg/kg/day when iodine intake was adequate and 0.01-0.03 mg/kg/day when iodine intake was inadequate.[70]	Incidentally these references cited are secondary, on examining the primary reference it is evident that thyroid hormone elevation is observed only following exposure to high fluorine concentration. i.e., Consumption of drinking water with elevated fluorine content (122 ± 5 umol/L) Bachinskii et al. 1985; Susheela et al. 2005: 1.1 to 14.3 mg F/L (~ 1.1-14.3 PPM). Moreover these studies have evaluated thyroid hormone levels in the context of iodine deficiency, which may have direct effects on thyroid hormone levels. Moreover none of the studies have shown evidence towards the fluoride levels in drinking water influencing thyroid function either acutely or chronically. Thyroid hormone effects on cardiac function is well known due to its direct influence on cardiomyocyte metabolism, however linking this to fluoride levels in drinking water is not supported with scientific evidence. Interestingly EPA-USA (references cited by the author) determined that the maximum amount of fluoride allowed in drinking water is 4.0 milligrams per litre (4 PPM). In the EPA statement it is clearly indicated that at 4PPM or lower concentration thyroid function is not affected.
Page 12	Fluoride has been implicated in disturbing the functionality of calcium, both directly[85] and indirectly in interaction with Vitamin D.[86]	References 85 and 86 don't support this statement. There is no evidence that calcium or Vit D levels are effected by fluoride levels in drinking water @ < 4 PPM
Page 12	In high calcium waters most of the fluoride is excreted while in low calcium waters the majority of fluoride is absorbed; resulting in elevated blood plasma fluoride levels, and retention of fluoride in various organs of the body.[89]	This is again not a peer-reviewed literature and doesn't have any experimental evidence to support the statement made.
Page 12	This view is supported by Krishnamachari in his review[91] when he found that In the presence of inadequate calcium, fluoride directly or indirectly stimulates the parathyroid glands, causing secondary hyperparathyroidism leading to bone loss.	This reference related to endemic fluorosis, which is several folds higher in fluoride exposure (> 5 PPM) compared to fluoride levels in drinking water in Ireland.
Page 12	Fluoride is known to be an inhibitor of enzymatic activity and research has identified fluoride as an inhibitor of	This inhibitory effect of fluoride is observed only at a very high concentration. i.e., 0.7 to 1.0 mole fluoride/mole of enzyme subunit, and 1.7 moles

	homocysteine hydrolase.[97]	fluoride/mole of enzyme subunit from I and II, respectively. This concentration corresponds to 28000 ï 40000 PPM of fluoride for every mole of the enzyme subunit or 68000 PPM of fluoride for every mole of the enzyme subunit form I and II. Moreover there is no evidence that such high concentration of fluoride can accumulate in the intracellular space when given systemically to achieve the enzyme inhibition effects.
Page 13	Research published in 2010 demonstrated that fluoride also affects the aorta (main artery) and heart in ways that lead to increased heart attacks. [99,100] This confirms earlier studies showing that high blood-fluoride levels have an effect on body calcium, leading to calcification of the aorta and other arteries.[101,102]	Ref 99: study done in endemic fluorosis area. urine fluoride levels of fluorosis patients were significantly higher than control subjects (1.9 ± 0.1 mg/l vs. 0.4 ± 0.1 mg/l, respectively; Ref 100: study done in endemic fluorosis area. The urine fluoride levels of fluorosis patients were significantly higher than control subjects (1.9 ± 0.1 mg/l vs 0.4 ± 0.1 mg/l respectively; mean fluoride level in drinking water was 2.74 ± 0.64 mg/l in the above studies. Based on my review of the literature, there is no scientific evidence to support fluorination of drinking water at 0.7-0.9 PPM can influence elasticity of aorta and heart by promoting tissue calcification. Whitford et al., 2008, Archives of Oral Biology; have performed pharmacokinetics studies of orally administered fluoride at 0.7-6 PPM in drinking water. In this study the highest plasma concentration achieved was 7 micromole/L (< 0.3 PPM) by 1 hr post administration and this peak concentration dropped to below 2 micromole/L (< 0.08 PPM) by 3 hr post administration. This elegantly performed pharmacokinetics study indicates that blood fluoride levels following fluoride ingestion is very transient and rapidly distributed or eliminated. Hence it is unlikely that drinking of fluorinated drinking water (0.7-0.9 PPM) can result in blood fluoride concentration sufficient to influence arterial or cardiac calcification.
Page 13	Song et al, Observations on fluoorotic aorta sclerosis by two doimensional echo cardiography ñEndemic diseases Bulliten 5, 1990, (1) 91-93	Cannot access this literature. However the title of this reference suggests the study was performed in fluoride endemic areas, hence presuming the fluoride levels were much higher than 1 PPM
Page 13	Animal studies by Ebert et al.[103,104] demonstrated that fluoride exposure resulted in retarded development of heart muscle and inhibition of heart function in developing chic embyros.	5 -20 mM sodium fluoride (~ 200-800 PPM) was used in this study. This concentration is several log fold over the fluoride levels in drinking water and unlikely to be consumed by anyone in real world.
Page 13	Research undertake by Spratt[105] concluded that primary site of fluoride	20 mM sodium fluoride (~ 800 PPM). Enolase is present in many tissue and is not specific to

	action was enolase, which is distributed predominantly in the heart and skeletal muscles and is a biomarker for myocardial damage.	heart or skeletal muscles. Further enolase is not a reliable biomarker for myocardial damage, although its increase levels are detected following acute myocardial infarction. Since enloase is an enzyme of glycolytic pathway, any cell or tissue damage may contribute to its increase and hence it is non-specifically associated with many pathologic conditions. Fluoride is a competitive inhibitor of enolase substrate and this effect is selective to bacterial enolase hence accounting for utility of fluoride in preventing bacterial plaque/dental caries. Fluoride inhibits mammalian enolase at concentrations >0.4M (~16000 PPM), i.e., a concentration unlikely to be achieved systemically.
Page 13	In addition to enolase inhibition, fluoride is recognized as a potent inhibitor of non-specific phosphatases and mitochondrial adenosine triphosphatase.[106,107]	5-10 mM sodium fluoride (~ 200-400 PPM). Again as above the fluoride concentrations to inhibit these enzymes is very high and it is very unlikely to achieve these concentrations of fluoride systemically.
Page 13	Slater & Bonner[108] found succinic dehydrogenase to be the site of fluoride inhibition in the succinoxidase system of heart-muscle preparations.	9.7-40.5 mM sodium fluoride (~350-1600 PPM). It is very unlikely to achieve these concentrations of fluoride systemically.
Page 13	Turla et al.[110] observed that fluoride inhibits calmodulin	The referenced study doesn't use fluoride, hence the statement made by the author is not correct.
Page 14	Varol et al. examined the effect of fluoride exposure on cardiovascular system in a clinical setting. and observed that elastic properties of ascending aorta were impaired in patients with endemic chronic fluorosis and that chronic fluoride toxicity can cause aortic stiffness in patients as well as ventricular diastolic and global dysfunctions.[112,113] Furthermore Varol et al. found that fluoride toxicity can cause atherosclerosis at molecular level, as well as aortic stiffness and disturbed ventricular distensibility at clinical level. [114]	The reference cited are same as ref no 99 and 100 above. This study was performed in endemic fluorosis area. The urine fluoride levels of fluorosis patients were significantly higher than control subjects (1.9 ± 0.1 mg/l vs 0.4 ± 0.1 mg/l respectively; mean fluoride level in drinking water was 2.74 ± 064 mg/l in the above studies Ref no 114: study performed at toxic concentration of fluoride
Page 14	Research has also demonstrated that fluoride accumulates in aorta vascular walls and that a significant correlation exists between fluoride uptake and coronary calcifiation.[115]	The author has not understood this study; here fluoride uptake was used as a diagnostic assessment of calcium in the arteries. Radio-labelled fluoride transient uptake by tissues is an index of calcification; this doesn't mean that fluoride accumulates in the tissue itself. Hence the conclusions drawn by the author on this study is wrong.

Page 14	Fluoride's effects were evident at water levels of 0.2mg/L or more of Fluoride.[116,117]	The references cited are secondary and data doesn't correlate. There is no scientific evidence for fluoride induced adverse effects at its water levels of 0.2mg/L. In fact several studies have shown that fluoride content in drinking water at < 2 PPM is safe (EPA, USA reports)
Page 14	Fluoride damages the heart muscle, especially in subjects deficient in Vitamins A and D.[121] They further observed that fluoride decreases the energy building glycogen in the muscles,[122] and that it adversely affects the functions of the kidneys.[123]	This study was done in endemic fluorosis area. mean fluoride level in drinking water was 2.74 ± 064 mg/l.
Page 14	Exposure to low levels of fluoride was a contributory cause of sudden infant death syndrome (SIDS), particularly within the lower income communities where poor nutrition was already prevalent.[126,127] On the subject of infant deaths, researchers at the New York State University (Department of Epidemiology and Biostatistics,School of Public Health).documented that municipal water fluoridation causes more premature births, after controlling for age, race/ethnicity, neighbourhood poverty level, hypertension and diabetes.[128]	None of the references (126, 127 and 128) cited are peer-reviewed and hence the scientific validity of these reports is questionable.
Page 15	As noted in the review by Barbier et al[129] chronic inflammation is harmful and has an important role in the development of several chronic diseases such as diabetes and atherosclerosis, both of which contribute significantly to CHD.	This reference is reviewed above and cited multiple times with different reference number. In my opinion this author has over interpreted and extrapolated major content of his manuscript from this review article. It is important to note that the fluoride concentration used in most of the studies reported in this review was much above the drinking water levels of fluoride (explained in detail above).
Page 15-16	An article published by Ma et al.[130] (2012) investigated the effect of exposure to fluoride alone on inflammatory response in rabbit aorta.	Toxic concentrations of fluoride (50 mg/L NaF), (~50 PPM) was used in this study and hence is irrelevant to associate inflammation with fluoride levels in drinking water.
Page 16	Oxidative stress is a recognized mode of fluoride action.[139]	Ref 139 is a review article and doesn't provide any scientific evidence that Oxidative stress is induced by fluoride at concentrations present in drinking water.
Page 16	Researchers Varol et al. reported that in addition to fluoride exposure causing oxidative stress, it may have an important role in cardiovascular disease. [141]	Study done in endemic fluorosis area. The urine fluoride levels of fluorosis patients were significantly higher than control subjects (1.9 ± 0.1 mg/l vs 0.4 ± 0.1 mg/l respectively; mean fluoride level in drinking water was 2.74 ± 064 mg/l (>2 PPM)

Page 16	In addition endothelial dysfunction and vascular disorders have been associated with fluoride exposure in humans.[144,145]	Both the reference doesn't show any evidence to support the statement made by the author.
Page 16	According to Barbier et al. *"the data suggest an important role played by factors related to oxidative stress and vascular inflammation, providing future directions for research into the cardiovascular effects of fluoride exposure."* [146]	This reference is reviewed above: the fluoride concentration used in most of the studies reported in this review was much above the drinking water levels of fluoride (explained in detail above).
Page 17	Professor Takamori's research team observed that children with dental fluorosis have a higher incidence of heart damage and an increase in abnormal heart rhythm than those without fluorosis.[151] These observations have been supported by studies conducted by Wang et al. in China.[152]	These studies are performed in fluoride endemic areas wherein fluoride concentration in drinking water is very high (> 4 PPM)

I have only reviewed the reference relevant to effects of fluoride on biological function. (The references related to epidemiological data was excluded).

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