Bull. Environ. Contam. Toxicol. (2001) 67:672–679

DOI: 10.1007/s00128-001-0176-x

Bulletin of Environmental Contamination and Toxicology

Cytotoxic and Cytoprotective Effects of Selenium on Bluegill Sunfish (*Lepomis macrochirus*) Phagocytic Cells *In Vitro*

S. Palchaudhuri,[1] A. Raymond,[2] E. A. Carlson,[2] Y. Li,[2] J. T. Zelikoff[2]

[1] Boston University, 1019 Commonwealth Avenue, Boston, MA 02215, USA
[2] New York University School of Medicine, 57 Old Forge Road, Tuxedo, NY 10987, USA

Received: 20 February 2001/Accepted: 26 June 2001

A major cause of degenerative diseases in aquatic environments is oxidative stress (Pryor 1986; Kelly et al. 1998). In addition to that which results from stressors associated with contaminated environments, 1-5% of reactive oxygen intermediates (ROI), [i.e., superoxide ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^{\cdot}$)] escape from the electron transport chain and have the potential to damage cell membranes, DNA, and proteins (Punchard and Kelly 1996). The greatest damage to cells is caused by H_2O_2 since it is converted to the highly-active OH^- radicals by reaction with either $O_2^{\cdot -}$ (i.e., Haber-Weiss reaction) or ferrous iron via the Fenton reaction (Pryor 1986). While research has typically focused upon mammalian species to elucidate mechanisms of oxidant-induced cell damage, it is becoming obvious that oxidative stress also affects contaminant-exposed aquatic organisms, and that both biological systems exhibit similar toxicological and adaptive responses to oxidative injury (DiGiulio et al. 1989; Winston and DiGiulio 1991; Kelly et al. 1998; Palace et al. 1998).

To prevent oxidative damage to cellular components, enzymatic and non-enzymatic antioxidant defenses are available within the cell to scavenge ROIs (Kelly et al. 1998). The activity of the extracellular and cytosolic forms of glutathione peroxidase (GSHPX), a critical enzyme with antioxidant properties that catalyzes the reduction of both organic peroxides and H_2O_2 by using reduced glutathione (GSH) to produce oxidized glutathione and water is dependent upon the essential trace element selenium (Se) (Kiremidijian-Schumacher and Stotzky 1987; Maier and Knight 1994; Kelly et al. 1998). Accordingly, inadequate levels of Se can lead to a reduction in GSHPX activity and, consequently, to a decreased ability to degrade H_2O_2 (Kelly et al. 1998). Deficient GSHPX and increased H_2O_2 levels have both been linked to auto-oxidation of cell membranes, microtubular and DNA damage, impairment of immune cell function and bactericidal activity (Spielberg et al. 1979; McCallister et al. 1980). Thus, homeostatic regulation of optimal levels of Se appears critical for protecting tissues from H_2O_2-induced oxidative damage and for maintaining overall health.

While Se is essential for the catalytic activity of many enzymes, it can also present a potential hazard at the cellular and organismal level (Medina and Oborn 1984; Seko et al. 1996). Selenium, long-recognized as a toxic element that can cause blind stagger disease in farm animals and poisoning in humans (Seko et al. 1996), can have potent cytotoxic effects by reacting with sulfhydryl groups to produce biologically-active ROI (Gaberg et al. 1988). Thus, depending upon Se concentration and the oxidant/antioxidant status of the cell, Se can play a dual role as a protective antioxidant and as a potentially toxic pro-oxidant.

Correspondence to: J. T. Zelikoff

An *in vitro* study using bluegill sunfish (*Lepomis macrochirus*) kidney phagocytes was undertaken to investigate the effects of Se on H_2O_2-induced oxidative injury because: more information is needed to better understand the paradoxical nature of Se in vertebrate systems and the delicate balance which exists between oxidant-induced stress and the antioxidant defense system; threshold levels for dietary Se in fish are often exceeded in contaminated aquatic environments (Hilton et al. 1980); and, phagocytic cells from both teleost and mammalian species are sensitive to the effects of Se (Low and Sin 1996). This study will also provide baseline knowledge needed to evaluate the applicability of the bluegill cell culture system as a model for assessing the impact of aquatic pollution and determining the effects of Se on higher vertebrates.

MATERIALS AND METHODS

Bluegill (*Lepomis macrochirus*), originally obtained from the United States Army Center of Environmental Health Research (Ft. Detrick, MD), were used as a source of kidney phagocytic cells. Briefly, fish were sacrificed, the head kidney tissue aseptically-removed, and single cell suspensions prepared as described for Japanese medaka (Zelikoff et al. 1996). Recovered phagocytic cells were suspended in L-15 media (Sigma, St. Louis, MO) supplemented with 5% bluegill serum and 1% L-glutamine and kept on ice until used (not more than 1 hr later).

To determine the effects of Se (administered as sodium selenite; Na_2SeO_3) on cell viability, 5 x 10^6 kidney phagocytes (in 100 μL supplemented L-15) were placed into wells of a 96-welled microtiter plate and after washing attached cells were exposed to either 0.5, 1.0, 10, 100, or 1000 μM SeO_3^{2-} (in serum-free L-15 media). Following exposure for 48 h at 30°C, the media was aspirated, and unattached cells counted using a hemocytometer. The viability of both attached and unattached cells were then assessed by trypan blue exclusion. Effects of H_2O_2 on phagocyte viability was determined in a manner identical to that described for SeO_3^{2-}, except that cells were exposed for only 2 h and the H_2O_2 concentrations tested ranged between 0.1 and 1000 mM. Chemical concentrations and exposure durations which resulted in less than 50% cell lethality were selected from previously-performed range-finding experiments.

The effects of SeO_3^{2-} pre-treatment upon H_2O_2-induced changes in intracellular $O_2^{\cdot-}$ production and phagocytic activity were determined by incubating 6 x 10^5 attached phagocytes with either 0.5, 1.0 or 10.0 μM SeO_3^{2-} (in serum-free media) for 48 h followed by a 2 h treatment of washed cells with 0, 100 or 1000 mM H_2O_2. Following exposure to both agents, detached cells were aspirated, counted, and viability determined by trypan blue exclusion.

Intracellular $O_2^{\cdot-}$ production was determined colorimetrically by reduction of nitroblue tetrazolium as previously-described (Zelikoff et al. 1996; Barron et al. 2000). Unstimulated and phorbol myristate acetate (PMA; Sigma, St. Louis, MO)-stimulated $O_2^{\cdot-}$ production was determined from the difference in optical density (at 630 nm) in wells containing superoxide dismutase from those wells without the enzyme. While PMA is not normally found within a host, it is commonly-used in *ex vivo* studies as a model PKC agonist to stimulate ROI production by both mammalian and fish phagocytes (Zelikoff et al. 1996; Froemming and O'Brien 1997). The final concentration of intracellular $O_2^{\cdot-}$ (nmoles/cell number) was adjusted to account for cell detachment due to treatment exposures. Phagocytic activity, as measured by changes in phagocytic index [(PI = number of cells that engulfed particles/total number of cells counted) x 100] and

phagocytic capacity [(PC = number of cells containing ≥ 3 particles per cell/total number of cells containing latex particles) x 100], was determined by incubating 5 x 10^5 attached kidney cells with bluegill serum-opsonized latex particles (3 μm, Duke Scientific, Palo Alto, CA) for 3 h (at 30°C) at a final particle to cell ratio of 200:1. Following incubation with particles, attached cells were washed, fixed in 2.5% cold glutaraldehyde (in phosphate-buffered saline; pH 7.4), immersed in methylene chloride (Fisher Scientific, Fair Lawn, NJ) for 3 min to remove non-ingested particles, and stained with Diff Quick (Fisher Scientific). Controls for each experiment consisted of cells alone and those exposed only to SeO_3^{2-} or H_2O_2 alone at each of their respective tested concentrations.

Statistical significance between and among groups was determined using a one-way analysis of variance (ANOVA) followed by Fisher post-hoc testing when appropriate. Significant differences were determined at $p < 0.05$.

RESULTS AND DISCUSSION

Findings from these studies indicated that *in vitro* exposure of bluegill kidney phagocytic cells to SeO_3^{2-} concentrations of 0.1, 1.0, 100 or 1000 μM for 48 h dose-dependently reduced cell viability. While exposure of cells to 1 μM SeO_3^{2-} reduced viability by 12%, viability dropped to 44% of control following incubation with the highest SeO_3^{2-} concentration; treatment for 24 h with SeO_3^{2-} at any of the tested levels had no effect upon cell survival. While Se is an essential trace element critical for normal cell function, it is also known to have potent toxic effects for exposed experimental animals (Shamberger 1983) and cell systems *in vitro* (Kitahara et al. 1993). For example, *in vitro* exposure to high concentrations of SeO_3^{2-} has been shown to be toxic for mammalian hepatocytes and immune cells (Kitahara et al. 1993; Sun et al. 1995a). Selenite has also been shown to produce toxicity in rainbow trout (*Onchorynchus mykiss*) receiving concentrations above the recommended dietary requirement (Hilton et al. 1980). In mammalian systems, cell lethality following exposure to excess SeO_3^{2-} is thought to be due to the production of $O_2^{\cdot -}$ and other ROIs which result in increased levels of lipid peroxidation and DNA strandbreaks (Kitahara et al. 1993).

Treatment of bluegill kidney phagocytes for 2 h with H_2O_2 concentrations between 0.1 and 1000 mM, like, SeO_3^{2-}, reduced cell viability in a dose-dependent manner. However toxic effects of H_2O_2 were far less dramatic than those produced by SeO_3^{2-}; while exposure to 10 μM SeO_3^{2-} reduced phagocyte viability by 44%, exposure to H_2O_2 at a 1000-fold greater concentration (i.e., 1000 μM vs. 1000 mM, respectively) decreased cell viability by only 14% (compared to control). This effect may be related to the shorter exposure duration employed.

Unstimulated intracellular $O_2^{\cdot -}$ production by attached kidney phagocytes increased in parallel (compared to the untreated control) with increasing SeO_3^{2-} concentrations; incubation with H_2O_2 alone increased $O_2^{\cdot -}$ production in a dose-dependent manner (Figure 1A). At approximately equitoxic concentrations (i.e., SeO_3^{2-} at 1.0 μM and H_2O_2 at 1000 mM), administration of SeO_3^{2-} or H_2O_2 alone increased unstimulated $O_2^{\cdot -}$ production by approximately 45- and 28-fold, respectively, above untreated control levels. This suggests that while both SeO_3^{2-} and H_2O_2 alone can induce oxidative stress in resting bluegill kidney phagocytes, SeO_3^{2-} is a more potent oxidant-inducer than H_2O_2 in this cell system. Other *in vitro* and *ex vivo* studies, have also demonstrated the ability of SeO_3^{2-} to

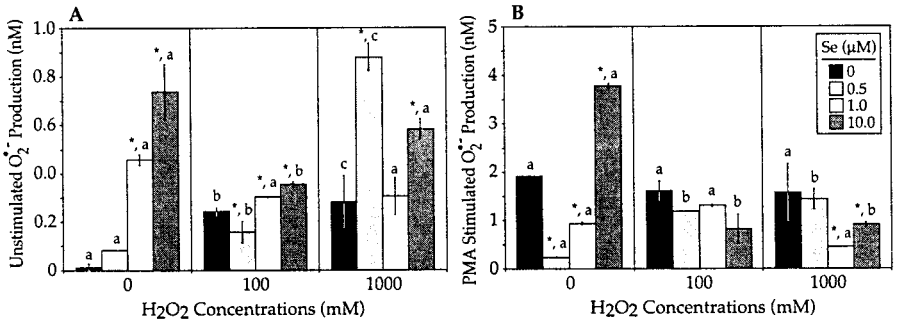

Figure 1. Effects of SeO_3^{2-} pre-treatment on H_2O_2-induced unstimulated (A) and PMA-stimulated (B) intracellular $O_2^{\cdot-}$ production by bluegill sunfish kidney cells. Values are means ± SEM for four individual fish.
*Within each H_2O_2 exposure group, value is significantly different from SeO_3^{2-}-free sample at $p < 0.05$.
a, b, c: At each given SeO_3^{2-} concentration, those with dissimilar letters are significantly different from each other at $p < 0.05$.

stimulate free radical production (Kiremidjian-Schumacher and Stotzky 1987; Kitahara et al. 1993; Low and Sin 1996). For example, incubation of blue gourami (*Trichogaster trichopterus*) head kidney phagocytes with SeO_3^{2-} at 4 ppm significantly increased ROI production compared to controls (Low and Sin 1996). In addition, isolated rat hepatocytes exposed to SeO_3^{2-} concentrations between 100 and 200 μM produced increased amounts of lipid peroxidation products (Kitahara et al. 1993); ROI-mediated oxidation of SH compounds, primarily GSH, were thought to be a key factor in the observed SeO_3^{-2}-induced cytotoxicity (Kitahara et al. 1993).

In general, pre-treatment of H_2O_2-exposed bluegill cells with SeO_3^{2-} for 48 hr increased unstimulated $O_2^{\cdot-}$ formation above that produced by the same concentration of H_2O_2 alone; in a single case (i.e., pre-treatment with 0.5 μM SeO_3^{2-} prior to treatment with 100 mM H_2O_2), this pre-incubation reduced inducible $O_2^{\cdot-}$ production (Figure 1A). Given that the toxicity of peroxidative compounds has been shown to be exacerbated under Se-deficient conditions (Kiremidjian-Schumacher and Stotzky 1987), and that $Se3O2^-$ pre-treatment ameliorated H_2O_2- and ^{60}Co-induced cell damage in exposed mouse lymphocytes (Sun et al. 1995b), the results from this part of the study were somewhat unexpected. Although $Se3O2^-$-related increases in unstimulated $O_2\cdot$ production were observed for each given H_2O_2 concentration tested, it is interesting to note that addition of H_2O_2 modulated $O_2^{\cdot-}$ formation produced in cells treated only with SeO_3^{2-}; treatment of cells previously-exposed to 0.5 μM SeO_3^{2-} with 100 and 1000 mM H_2O_2 dose-dependently increased $O_2^{\cdot-}$ production (compared to that produced by cells exposed to SeO_3^{2-} alone). While similar effects were not observed for cells pre-treated with 1 μM SeO_3^{2-}, cells treated with 10 μM SeO_3^{2-} and then exposed to 100 mM H_2O_2 produced 52% less $O_2^{\cdot-}$ than cells treated with SeO_3^{2-} alone. Further studies are needed to better understand these findings. However, results clearly support previously-published mammalian literature demonstrating the ability of SeO_3^{2-} to be both protective and toxic by acting as either an anti-oxidant or pro-oxidant, respectively.

In contrast to that observed for unstimulated $O_2^{\cdot-}$ production, exposure to H_2O_2 alone had no effect upon ROI production by fish phagocytes stimulated *in vitro*

with PMA (Figure 1B). However, exposure of PMA-stimulated bluegill phagocytes to SeO_3^{2-} dramatically-altered $O_2^{\cdot -}$ production; incubation of naive fish cells with 0.5 and 1.0 μM SeO_3^{2-} for 48 h significantly reduced $O_2^{\cdot -}$ production (compared to untreated control), while treatment with 10 μM SeO_3^{2-} enhanced production approximately 50% above PMA-stimulated control cells (Figure 1B). Augmented production of $O_2^{\cdot -}$ following *in vitro* and/or *in vivo* treatment with high doses of SeO_3^{2-} has also been observed in mammalian studies (Kitahara et al. 1993; Seko et al. 1996; Low and Sin 1996) and is thought to occur as a result of SeO_3^{2-} reduction to selenide (via GSH and/or glutathione reductase) which then supplies an electron to oxygen, thereby generating $O_2^{\cdot -}$. Selenite-associated suppression of $O_2^{\cdot -}$ has also been observed, but usually only as a result of Se deficiency (Aziz et al. 1984). How Se deficiency might relate to treatment of fish phagocytes with low doses of SeO_3^{2-} is not yet apparent.

Exposure of bluegill phagocytes to H_2O_2 after exposure to SeO_3^{2-} appeared, in certain cases, to partially relieve the immunotoxic effects produced by SeO_3^{2-} treatment alone; PMA-stimulated $O_2^{\cdot -}$ production, significantly depressed by exposure to 0.5 μM SeO_3^{2-} alone, began to approach the untreated control value following exposure to either H_2O_2 concentration. While effects produced by 1.0 μM SeO_3^{2-} alone were unaffected by exposure to H_2O_2, incubation with H_2O_2 significantly reduced the enhancement produced by treatment with 10 μM SeO_3^{2-}. Also of interest is the fact that $O_2^{\cdot -}$ production by cells exposed to 1000 mM H_2O_2 was significantly reduced by pre-treatment with the two highest Se concentrations (compared to cells treated with H_2O_2 alone). Thus, it appears that the concentration of both SeO_3^{2-} and H_2O_2 are critical for determining whether overall effects are toxic or protective against oxidative stress. Interestingly, transition metals such as zinc and cadmium have also been shown to impart protective effects upon H_2O_2-induced toxicity; protective effects were attributed, at least in part, to increased production of metallothionein and/or GSH by the *in vitro* exposed teleost hepatoma cell line (Schlenk and Rice 1998).

Chemical-induced cytotoxicity can often be determined by examining phagocytic activity (as measured by changes in PI and/or PC). *In vitro* treatment with either H_2O_2 or SeO_3^{2-} alone significantly reduced (with one exception) the ability of bluegill kidney phagocytes to engulf serum-opsonized latex particles (Figures 2A and B). Exposure to increasing H_2O_2 concentrations, in the absence of SeO_3^{2-} pre-treatment, reduced both the percentage of cells engulfing particles (i.e., PI) and the total number of particles ingested (i.e., PC); while effects upon PC (Figure 2B) appeared to be dose-dependent, those on PI (Figure 2A) were less consistent. Like those effects produced by H_2O_2 on PC, exposure to SeO_3^{2-} alone at 0.5, 1.0, and 10.0 μM also reduced the PC of exposed bluegill cells; although PI was also significantly reduced, this effect occurred only following treatment with the lowest and highest SeO_3^{2-} concentrations. While the mechanism by which SeO_3^{2-} might act to reduce the phagocytic activity of bluegill cells is not yet known, it has been speculated that high concentrations of SeO_3^{2-} decrease cellular GSH levels which in turn can lead to the production of excess free radicals and a decreased ability of the cells to degrade H_2O_2 (Kitahara et al. 1993); decreased GSH redox cycle activity can lead to auto-oxidation of cellular membranes and cytoskeletal structures important for phagocytosis (Kiremidjian-Schumacher and Stotzky 1993).

Interestingly, SeO_3^{2-} pre-treatment modulated the suppressive effects on PI produced by exposure to 100 mM H_2O_2 alone (Figure 2A). Exposure of bluegill

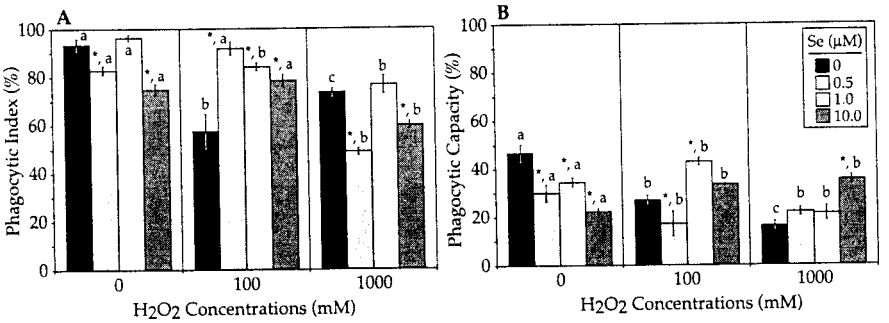

Figure 2. Effects of SeO_3^{2-} pre-treatment on phagocytic index (A) and phagocytic capacity (B) of bluegill sunfish kidney cells. Values are means ± SEM of four individual fish.
*Within each H_2O_2 exposure group, value is significantly different from SeO_3^{2-}-free sample at $p < 0.05$.
a, b, c: At a given SeO_3^{2-} concentration, those with dissimilar letters are significantly different from each other at $p < 0.05$.

phagocytes to 0.5 μM SeO_3^{2-} completely ameliorated the effects produced by 100 mM H_2O_2 alone and restored phagocytic activity back to that level measured in cells not receiving H_2O_2. On the other hand, pre-treatment with both 0.5 and 10.0 μM SeO_3^{2-} worsened the effects on PI produced by sequential exposure to 1000 mM H_2O_2. Taken together, these findings suggest that low-doses of SeO_3^{2-} act to protect fish phagocytes against cell injury produced by relatively low concentrations of H_2O_2; at higher H_2O_2 concentrations, pre-treatment with SeO_3^{2-} enhances toxicity over and above that produced by each individual agent alone. Measurements of PC, often a more sensitive indicator of toxic effects than PI, also demonstrated that SeO_3^{2-} pre-treatment could both exacerbate and protect against H_2O_2-induced phagocytic injury (Figure 2B). Effects of SeO_3^{2-} pre-treatment on H_2O_2-induced alterations in PC appeared dependent upon the concentration of both agents. While exposure to 1.0 or 10.0 μM SeO_3^{2-} increased (compared to cells treated with H_2O_2 alone) the PC of cells exposed to 100 mM H_2O_2 by 40 and 23%, respectively, incubation with the lowest SeO_3^{2-} concentration appeared to worsen the effects. Exposure of cells to 1000 mM H_2O_2 reduced the PC from 46% (in the untreated control group) to 16%. Exposure to increasing SeO_3^{2-} concentrations appeared to reduce this effect; pre-treatment with the highest SeO_3^{2-} concentration afforded partial protection against H_2O_2-induced toxicity and to some extent restored PC back to that observed in the untreated control (i.e., 35 vs. 46%, respectively). Similar protective effects of SeO_3^{2-} have also been observed for H_2O_2-damaged mammalian immune cells (Sun et al. 1995a). In these studies, *in vitro* administration of 100 ppm SeO_3^{2-} prior to treatment with either 0.5 or 1.0 ppm H_2O_2 prevented the abrogation of concanavalin A-stimulated lymphoproliferation produced by exposure to H_2O_2 alone. Interestingly, effects of SeO_3^{2-} pre-treatment on H_2O_2-exposed mouse lymphocytes appeared to be dependent upon H_2O_2 concentration; SeO_3^{2-} pre-treatment only partially restored lymphocyte function when H_2O_2 concentrations exceeded 1.0 ppm. Treatment of the mouse lymphocytes with SeO_3^{2-} immediately after H_2O_2 treatment also proved effective for protecting against H_2O_2-induced injury.

Results from this study demonstrate the ability of SeO_3^{2-} to act as a pro-oxidant, resulting in the formation of excess $O_2^{\cdot-}$ by unstimulated fish phagocytes.

Phagocytic activity was also affected by SeO_3^{2-} treatment, particularly at the highest concentration tested; however, these effects were not as dramatic as those produced by H_2O_2 alone. Effects of H_2O_2 on phagocytosis were not that surprising given that cytoskeletal structures are known to be altered by the presence of a strong oxidizing micro-environment (Kiremidjian-Schumacher and Stotzky 1987). Moreover, if SeO_3^{2-} can deplete GSH levels in teleost cell systems, as is hypothesized for mammals, this could provide a partial explanation as to how SeO_3^{2-} exposure might lead to reductions in phagocytic activity by bluegill immune cells.

These findings, like those observed in mammals, also demonstrated that *in vitro* exposure of immune cells to SeO_3^{2-} can modulate the effects of H_2O_2, in some cases protecting against oxidative stress factors while, under other conditions, exacerbating oxidant-induced immune cell injury. Interestingly, known GSH-reducing agents, such as buthionine sulfoximine has also been shown to exacerbate H_2O_2-induced toxicity in fibroblasts from the same fish species used herein (Babich et al. 1993). Clearly, more studies need to be performed so as to better understand the paradoxical nature of Se as an essential nutrient and toxic agent. Taken together, results from these studies demonstrate the usefulness of fish for studies in oxidative stress toxicology. Moreover, given that the aquatic environment provides a sink for many chemical contaminants that have the potential to cause oxidative injury, a better understanding of oxidative stress in aquatic organisms is critical. Studies in fish will not only increase our knowledge of oxidative stress mechanisms, but expand the ability to extrapolate the phenomenon across vertebrate species.

Acknowledgments. Supported by the United States Army Center of Environmental Health Research Contract No. DAMD-60-1-8109.
Disclaimer: The views, opinions, and/or findings contained in this report are those of the author(s) and should not be construed as official Department of the Army position, policy, or decision, unless so designated by other official documentation. Research was conducted in compliance with the Animal Welfare Act, and other Federal statues and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals (NRC 1996) in facilities that are fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, International.

REFERENCES

Aziz ES, Klesius PH, Frandsen JC (1984) Effects of selenium on polymorphonuclear leukocyte function in goats. American J Vet Res 45:1715-1718

Babich H, Palace MR, Stern A (1993) Oxidative stress in fish cells: *In vitro* studies. Arch Environ Contam Toxicol 24:173-178

Barron MG, Anderson M, Beltman D, Podrabsky T, Walsh W, Cacela D, Lipton J, Teh ST, Hinton SJ, Zelikoff JT, Dikkeboom AL, Lasee BA, Woolley SK, Tillitt DE, Holey M, Bouchard P, Denslow N (2000) Association between PCBs, liver lesions, and biomarker responses in adult walleye (*Stizostedium vitreum vitreum*) collected from Green Bay, Wisconsin.. J Great Lakes Res 3:156-170 (2000)

DiGiulio RT, Washburn PC, Wenning RJ, Winston GW, Jewell CS (1989) Biochemical responses in aquatic animals: A review of determinants of oxidative stress. Environ Toxicol Chem 8:1103-1123

Froemming GR and O'Brien NM (1997) U937 cells as model to study the effect of phyochemicals on superoxide anion production. Nutr. Res. 17:1091-1103
Gaberg P, Stahl A, Warholm M, Hogberg J (1988) Studies of the role of DNA fragmentation in selenium toxicity. Biochem Pharmacol 37:3401-3406
Hilton JW, Hodson PV, Slinger SJ (1980) The requirement and toxicity of selenium in rainbow trout (*Salmo gairdneri*). J Nutr 11:2527-2535
Kelly SA, Havrilla CM, Brady TC, Abramo KH, Levin ED (1998) Oxidative stress in toxicology: Established mammalian and emerging piscine model systems. Environ Health Perspect 106:375-384
Kiremidjian-Schumacher L, Stotzky G (1987) Selenium and immune responses. Environ Res 42:277-303
Kitahara J, Seko Y, Imura N (1993) Possible involvement of active oxygen species in selenite toxicity in isolated rat hepatocytes. Arch Toxicol 67:497-501
Low KW, Sin YM (1996) *In vivo* and *in vitro* effects of mercuric chloride and sodium selenite on some non-specific immune responses of blue gourami, *Trichogaster trichopterus* (Pallus). Fish Shellfish Immunol 6:351-362
Maier KJ, Knight AW (1994) Ecotoxicology of selenium in freshwater systems. Rev. Environ. Contam. Toxicol. 134:31-48
McCallister J, Harris RE, Baehner PL, Boxer LA (1980) Alteration of microtubule function in glutahione peroxidase-deficient polymorphonuclear leukocytes. J Reticuloendothelial Soc 27:59-66
Medina D, Oborn CJ (1984) Selenium inhibition of DNA synthesis in mouse epithelial cell line YN-4'. Cancer Res 44:4361-4365
Palace VP, Baron CL, Klaverkamp JF (1998) An assessment of Ah-inducible phase I and phase II enzymatic activities and oxidative stress indices in adult lake trout (Salvelinus namaycush) from Lake Ontario and Lake Superior. Aquat. Toxicol. 42:149-168
Pryor WA (1986) Oxyradicals and related species: Their formation, lifetimes and reactions. Ann Rev Physiol 48:657-667
Punchard NA, Kelly FJ, (eds). (1996) Free Radicals: A Practical Approach. Oxford:IRL Press
Schlenk D, Rice CD (1998) Effect of zinc and cadmium treatment on hydrogen peroxide-induced mortality and expression of glutathione and metallothionein in a teleost hepatoma cell line. Aquat. Toxicol. 43:121-129
Seko Y, Kitahara J, Imura N (1996) Hydroxyl radical generation by selenium compounds as a possible mechanism of selenium toxicity. Proc Third Intl World Congress Biomed Sci, Tsukuba, Japan pp. 5
Shamberger RJ (1983) Toxicity of selenium. In: Shamberger RJ (ed) Biochemistry of Selenium. Plenum, New York pp. 185-206
Spielberg SP, Boxer lA, Oliver JM, Allen JM, Schulman JD (1979) Oxidative damage to neutrophils in glutathione synthetase deficiency. British J Haematol 42:215-223
Sun E, Huibi X, Liu Q, Zhou J, Zuo P, Wang (1995a) Effect of selenium in recovery of immunity damaged by H_2O_2 and ^{60}Co radiation. Biol Trace Elem Res 48:239-250
Sun E, Xu H, Liu Q, Zhou J, Zuo P, Wang J (1995b) The mechanism for the effect of selenium supplementation on immunity. Biol Trace Elem Res. 48:231-238
Winston GW, DiGiulio, RT (1991) Prooxidant and antioxidant mechanisms in aquatic organisms. Aquat. Toxicol. 19:137-161
Zelikoff JT, Wang W, Islam N, Twerdok LE, Curry M, Beaman, J, Flescher E (1996) Assays of reactive oxygen intermediates and antioxidant enzymes: Potential biomarkers for predicting the effects of environmental pollution. In: Ostrander GK (ed). Aquatic Toxicology. CRC Lewis Publ Inc, Boca Raton, FL, pp. 287-305