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Arsenic-induced responses in freshwater teleosts

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Abstract The environment is currently polluted by thousands of chemicals or xenobiotics introduced into the environment by man to meet the demands of the modern era. Every day we encounter this negative side of human civilization, but have done little to lessen the rate of pollution. Although the entire biosphere is polluted it is water resources that are the most polluted because water is the ultimate sink for many contaminants. Thus, fish are the most vulnerable of all animal species. They are helpless because they cannot avoid the polluted habitat and face this contamination by default. Nevertheless, fish are found to survive under extreme conditions when their natural habitat has been compromised to a great extent. However, fish are highly sensitive to small environmental changes and their populations gradually dwindle if pollution continues unabated. However, we know that there are instances when water is cleaned and the rate of repopulation by different fish species has gained momentum, restoring the ecological balance. Thus, fish are considered reliable bioindicators of water pollution and fish ecotoxicology has received much attention in recent years, and fish toxicology has been able to defend a significant position in the arena of xenobiotics research over the years. This review deals with some of the major intoxication and detoxication signals manifested by fish exposed to arsenic (As), which is presently one of the most worrying metalloids in water pollution.

Keywords Arsenic · AChE–ACh system · Detoxication · GSH–GST system · HSP · Intoxication · Lipid peroxidation · Metallothionein · ROS

Abbreviations

ACh	Acetylcholine
AChE	Acetylcholinesterase
ACOX	Acyl co-A oxidase
CD	Conjugated dienes
GPx	Glutathione peroxidase
GR	Glutathione reductase
GSH	Glutathione reduced
GSSG	Glutathione oxidized
GST	Glutathione-S-transferase
HSP -70	Heat shock protein 70
LPO	Lipid peroxidation
MDA	Malondialdehyde
MT	Metallothionein
NAC	N-acetyl-cysteine
ROS	Reactive oxygen species

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SOD	Superoxide dismutase
–SH	Thiol groups
TBARS	Thiobarbituric acid reactive substances

Introduction

Metals and metalloids have the greatest disease-causing potential when they accumulate in the body. Therefore, their distribution kinetics is the prime factor in the evaluation of their toxicity. The aberrations that they cause include reproductive, developmental, immunological, and neurological toxicity, which are of increasing concern. Of these metals, the sulphydryl-reactive metals (arsenic, lead, cadmium, and mercury) are particularly sinister in that they interfere with a large number of crucial metabolic events and disrupt normal homeostasis in all lifeforms (Quig 1998). Among these, the pollution level of As (group VA) has reached an alarming level in many places in the world, including India (Chakraborty et al. 2003), especially West Bengal (Acharyya et al. 1999). Arsenic may undergo a variety of reactions in the environment, including oxidation-reduction reactions, ligand exchange, precipitation, and biotransformation (Welch et al. 1988). In aquatic systems, inorganic arsenic can occur in both As^{3+} and As^{5+} oxidation states. Both forms generally exist together, although As^{5+} predominates under oxidizing conditions (upper surface of water) and As^{3+} predominates under reducing conditions (lower water column and near sediments). The ratio of As^{3+} to As^{5+} depends on reactions that are influenced by Eh (the oxidation-reduction potential), pH, metal sulfide and sulfide ion concentrations, iron concentration, temperature, salinity, and the distribution and composition of the biota in an aquatic ecosystem (Wakao et al. 1988). Water samples from a number of lakes and estuaries therefore show measurable concentrations of methylated arsenic (equivalent to 1–59% of total arsenic) (Anderson and Bruland 1991), which is correlated with algal blooms (Nriagu and Pacyna 1988). Arsenic in its 3+ oxidation state is a carcinogen and can produce liver enlargement, cell degradation, necrosis, and fibrosis (Santra et al. 2000).

Aquatic ecosystem is at risk of contamination by arsenic from the leaching of inorganic arsenic compounds formerly used in pesticide sprays, from the combustion of arsenic-containing fossil fuels, and from the leaching of mine tailings and smelter runoff. Fishes are key species in an aquatic ecosystem and hence deserve a thorough attention in the surveillance of aquatic ecosystems. Reports on the effects of various pollutants strongly suggest that intoxication and detoxication processes analogous to those in mammals provide protection against xenobiotic damage in fish (Chatterjee 1983; Dalal 1989; Sarkar 1997). The present review will address some of the intoxication and detoxication signals induced by arsenic in teleosts.

The effect of arsenic in fish

Arsenic is graded as one of the most toxic elements to fish. Acute exposure can result in immediate death because of As-induced increases in mucus production, causing suffocation, or direct detrimental effects on gill epithelium. In fish, bizarre morphological alterations, as well as early neoplastic alterations are produced in the liver (Irwin et al. 1997). However, most of the data on the effects of arsenic on fish are based on acute toxicity tests, which measure fish mortality over 96 h. Some studies have also examined sublethal effects such as growth, avoidance behavior, and fertilization/hatching. Lima et al. (1984) exposed fathead minnow (*Pimephales promelas*) and flagfish (*Jordanella floridae*) to arsenite in 29-day and 31-day tests where growth was significantly reduced at concentrations of 4.3 mg l^{-1} and 4.12 mg l^{-1} As^{3+} for the two species, respectively. The no-observed-effect concentration (NOEC) based on growth was between 2.13 and 4.3 mg l^{-1} As^{3+} for *P. promelas* and between 2.13 and 4.12 mg l^{-1} As^{3+} for *J. floridae*. Growth of fingerlings of freshwater murrel (*Channa punctatus*) was significantly reduced by As_2O_3 at 7 mg l^{-1} As^{3+} during a 31-day test (Shukla et al. 1987). In 32-day tests on *P. promelas*, growth was found to be the most sensitive parameter (Spehar and Fiandt 1986). The avoidance threshold for golden shiner (*Notemigonus crysoleucas*) was 28 μg l^{-1} As^{3+} (as arsenite) in flow-through tests (Hartwell et al. 1989). Birge et al. (1978) exposed rainbow trout (*O. mykiss*) for eight days and largemouth bass (*Micropterus salmoides*) for 28 days to arsenite from fertilization to four days after hatching

and the LC_{50}s were found to be 0.54 and 42.1 mg l^{-1} As^{3+} for trout and bass, respectively.

Sodium arsenite has been used extensively as a herbicide for the control of mixed submerged aquatic vegetation in freshwater ponds and lakes; concentrations of 1.5 to 3.8 mg As^{3+} l^{-1} have usually been effective and are considered safe for fish (NAS, 1977). Recent data, however, have indicated that As^{3+} concentrations considered effective for aquatic weed control may be harmful to several species of freshwater teleosts, including bluegills (*Lepomis macrochirus*), flag fish, fathead minnows, and rainbow trout. Finfish exposed to 1–2 mg total As l^{-1} for 2–3 days may show one or more of several signs: hemorrhagic spheres on gills; fatty infiltration of liver; and necrosis of heart, liver, and ovary (NRCC 1978). In green sunfish (*Lepomis cyanellus*), there were changes in hepatocytes parallel to arsenic accumulation in the liver (Sorensen et al. 1985). Oral administration of sodium arsenate to estuary catfish (*Cnidoglanis macrocephalus*) and school whiting (*Sillago bassensis*) resulted in tissue accumulations of trimethylarsine oxide. Exposure to arsenic produced no measurable change in the gonads of freshwater fish, *Colisa fasciatus*, at the dose range of 2.0 mg l^{-1} within 15 or 30 days, whereas it produced marked alteration in both the organs at a higher dose range (14.0 mg l^{-1} As^{3+} oxide exposure for 30 days). Testicular changes included degeneration in the lobules, reduction in secretory cells and altered spermatogenesis associated with necrosis and pyknosis. Ovarian changes included decrease in the development of oocytes (stages II and III), reduction in the number and diameter of nucleoli, and increased number of atretic follicles (Shukla and Pandey 1984). The freshwater catfish *Clarias batrachus* exposed to arsenic showed an increased protein content in the liver, along with a decrease in dry weight and an increase in free amino acid and tissue permeability (Jana et al. 1986).

Numerous data are available on bioaccumulation, biomagnification and speciation of arsenic in fish and the effect of arsenic (especially As^{3+}) on fish reproduction, development, overall growth, and behavior. However, little information is available regarding the biochemical basis of such toxic effects or mechanisms involved in such processes. There is a need to investigate the mechanisms of action of this enigmatic metalloid on the cellular system, as it has been thoroughly studied in various animal models, cell lines, and in vitro systems. Taken together, the available data support the general hypothesis of involvement of cellular thiol, the generation of free radicals or reactive oxygen species, oxidative stress, antioxidant enzymes, metallothioneins, and activation of mitogen activated protein kinase (MAPK) pathways in the carcinogenic and anticarcinogenic action of Arsenic.

Toxic responses elicited by fish

Acetylcholinesterase–acetylcholine system

Weiss (1961) was probably the first to suggest that very low concentrations of organophosphates could be detected by measuring the degree of inhibition of fish brain acetylcholinesterase (AChE) and noted that fish may survive even when the enzyme activity is as low as 10–20% of normal. Acetylcholine (ACh) is an excitatory neurotransmitter that causes the transmission of nerve impulse through cholinergic synapses in vertebrates. It is interesting to note that a high level of AChE in any part of the brain of vertebrates is related to the acetylcholine level in these structures. Earlier reports on two air-breathing teleosts *Channa punctatus* and *Anabas testudineus* (Jash et a1. 1982; Jash and Bhattacharya 1983) could be related to the ACh level in the brain. Guhathakurta and Bhattacharya (1984) also corroborated that a definite inverse interrelationship exists between Ach content and AChE activity in different areas of the brain. Thus, the acetylcholine content is always found to be higher when the AChE activity is low, andregions that have a low ACh profile demonstrate higher enzyme activity.

The inhibitory action of arsenic on AChE is rather poorly studied (Tripathi et al. 1997; Stoytcheva et al. 1998). Arsenite is a well-known and potent inhibitor of enzymes that contain lipoic acid; the mechanism of inhibition is the formation of a cyclic dithioarsenite diester. It is remarkable that AChE is also readily inhibited by arsenite even, though this enzyme contains no lipoic acid and no sulfhydryl groups (Page and Wilson 1985). Roy et al. (2006) recently reported significant dose-dependent increase in AChE activity at 1/10 and 1/20 LC_{50} dosages of arsenic on day 1, a decrease on day 2 and regained activity on day 7 reaching the basal level on day 14. Moreover,

Roy et al. (2006) further demonstrated arsenic to be an agent of intoxication signals, exemplified by aberration in the brain AChE-ACh system, strongly correlating with the histoarchitectural damage recorded in the optic tectum.

Lipid peroxidation

The most typical reaction during reactive oxygen species (ROS)-induced damage involves the peroxidation of unsaturated fatty acids (Kappus 1987). Lipid peroxidation (LPO) is a free-radical chain reaction and is an attractive general mechanism that could explain the toxicity of various chemicals. It is primarily an outcome of oxidation and the formation of free radicals by peroxides and superoxides that are generated continuously in the living cells exposed to conditions of environmental stress. LPO alters the normal structural and functional properties of the cell, ultimately leading to cytotoxicity by dismantling the membrane structure in association with various adaptive reactions and changes in physiological status. LPO increases in response to various diseases and tissue damage is maximally observed in liver cells, which are more susceptible to stress-induced damage (Emerit and Chaudiere 1989). LPO is estimated by assessing the amount of thiobarbituric acid reactive substances (TBARS) in a sample, which is used as a measure for ROS-induced lipid peroxidation. TBARS, which include MDA and other such aldehydes and conjugated dienes (CD), are produced by LPO and are considered indicators of oxidative stress.

When compared with other vertebrates, fish (*Salmo trutta*) have similar lipid peroxidation level (Lopez-Torres et al. 1993) and increased amounts of TBARS have been found in fish under experimental conditions of exposure to xenobiotics. It has also been noted that TBARS level is species dependent. It has been reported that elasmobranchs have higher TBARS than marine teleosts and much higher values than freshwater teleosts (Filho 1996). LPO tends to be lower in herbivorous fish than in omnivorous species, correlating with lower glutathione peroxidase and catalase activities, although the herbivorous species have higher SOD activity (Radi et al. 1985). Experimental exposure of channel catfish (*Ictalurus punctatus*) to different concentrations of bleached kraft mill effluents did not increase lipid peroxidation in liver (Mather-Mihaich and DiGiulio 1991). TBARS remained unchanged in *Mugil* sp. from a polluted site together with low lipohydroxide levels compared to specimens from an unpolluted site (Rodriguez-Ariza et al. 1993). In contrast to these findings, sole from a polluted harbor showed high lipid peroxidation (DiGiulio et al. 1993).

Das et al. (1998) reported peroxidative damage in *A. testudineus* caused by chronic exposure to arsenic. In recent studies Roy et al. (2004) and Bhattacharya and Bhattacharya (2005) reported oxidative stress imposed by arsenic treatment in two fresh water teleost species, *C. punctatus* and *C. batrachus* exposed for 14 days to 1/10 and 1/20 LC_{50} As_2O_3, which caused significant elevation in MDA/TBARS as well as CD content in the liver. To see whether the observed increase in LPO has any relation to the generation of ROS, TBARS and CD contents were also measured in the antioxidant N-acetyl-cystamine (NAC)-preinjected fish exposed to arsenic. It was found that pretreatment with NAC could contain the increase in TBARS and CD at both exposure doses (Bhattacharya and Bhattacharya 2005).

Detoxication signals induced by arsenic

Detoxication by GSH–GST system

The detoxication machinery counters all toxicants that induce oxidative stress in a cell in a similar manner. The biotransformation requires specific functional groups that are required for subsequent metabolism by phase II enzymes. The phase II reactions are biosynthetic, involving conjugation of a xenobiotic with an endogenous compound. The biosynthetic reactions lead to conversion of hydrophobic moieties to easily excretable forms, thus increasing a cell's ability to remove the toxicant. The enzymes involved in the biotransformation reactions catalyze diverse types of reactions namely oxidation, reduction, epoxidation, deamination, hydroxylation, desulfuration, dehalogenation, and conjugation with endogenous compounds. The very elemental nature of metals and their diverse affinities for organic molecules in biological structures produce a bewildering array of biological effects, involving multiple target organs and systems, and diverse routes

(Hammond and Beliles 1984). Conjugation and sequestration of ionized metals from the surroundings is the strategy for detoxification of these metal protective biomolecules. The key enzymes of detoxication have been reported to be abundant in fish tissues (DiGiulio et al. 1989). Investigations reveal the presence of well-defined xenobiotic metabolism systems in fish, analogous to higher vertebrates, which are equally potent in the elimination of a multitude of xenobiotics (Chatterjee and Bhattacharya 1984; Sarkar 1997).

The role of GSH in protecting cells from metal toxicity is well documented. GSH has been recognized as an endogenous antioxidant that serves to protect against ROS injury. GSH reacts with metals non-enzymatically like other thiols (Chatterjee and Bhattacharya 1986; Dalal and Bhattacharya 1991; Sarkar 1997). Metals catalyze the autooxidation of thiols including GSH, at alkaline pH with a concomitant production of superoxide radicals. The overall reaction between GSH and metal is as follows (Albro et al. 1986):

$$6\ \mathrm{GSH} + \mathrm{O_2} + 2\ \mathrm{Me^{2+}} \rightarrow \mathrm{GSSG} + 2\ \mathrm{GS\text{-}Me\text{-}SG} + \mathrm{H_2O_2} + 4\mathrm{H^+}$$

Teleosts, as in mammals, possess high concentrations of GSH in their tissues. James (1987) demonstrated the presence of conjugates of GSH and mercapturic acid in fish bile after xenobiotic exposure. Exposure to metals has been shown to alter the GSH status in fish; chronic exposures to Pb, Cd, and Hg cause time- and dose-dependent increases in the hepatic GSH concentrations in various teleost species (Thomas and Wofford 1984), whereas acute exposure to Cd was found to reduce GSH levels (Sarkar 1997). In fish exposed to pollutants, both types of response may occur: firstly, a decrease in glutathione which is an acute reaction; secondly, an increase in glutathione concentration which is an adaptation to increased detoxification activity.

A decrease in glutathione was observed in climbing perch (*A. testudineus*) exposed to industrial pollutants after short- and long-term exposure (Chatterjee and Bhattacharya 1984), in the liver of bullhead (*Cottus gobio* L.) from a polluted site (Bucher et al. 1993) and in channel catfish exposed to bleached kraft mill effluents (Mather-Mihaich and DiGiulio 1991). The glutathione disulfide/glutathione ratio is a measure of the intracellular redox state, higher values indicating oxidative stress. An oxidized glutathione redox status was found in *Mugil* sp. from a polluted area (Rodriguez-Ariza et al. 1993). A higher-dose short-term exposure to Hg and Cd resulted in an activation of the GSH–GST system in *A. testudineus*, indicating that those xenobiotics were primarily detoxified by the GSH–GST system (Sarkar 1997).

Chronic exposure to low concentrations of Hg and Cd resulted in variable effects. While the hepatic GSH levels increased only slightly upon exposure to Hg, the levels were considerably higher in the case of Cd treatments, indicating Cd to be more toxic than Hg. Fish with an increased glutathione disulfide level appear prone to pollution-mediated damages and carcinogenesis (Hasspieler et al. 1994). Further, metals such as Hg and Cd that are able to induce metallothionein synthesis demonstrated an increase in GSH levels in both mammalian and piscine tissues (Das et al. 1998; Bose 1993; Sarkar 1997; Roy 2003). The requirement of glutathione for the protection of tissues is documented by the fact that toxicant-induced damages occur only if intracellular glutathione is depleted.

Glutathione and glutathione-S-transferase

It has been recently reported that, on treatment with arsenic, variations in the GSH content in the liver of *C. batrachus* (Bhattacharya 2004) and *C. punctatus* (Roy 2004) occurred in a biphasic manner. Concomitantly the response of GST was also found to be a biphasic one in these two teleosts exposed to 1/20 LC_{50} and 1/10 LC_{50} levels of As_2O_3. A probable increase in ROS, as indicated by an increase in LPO, could also lead to an increase in the GSSG content and subsequent redox imbalance through the alteration of the GSSG:GSH ratio. It was seen that the GSSG:GSH ratio in arsenic-exposed *C. batrachus* increased significantly at both doses, reached its peak on day 2, and like the LPO, showed a biphasic response. Pretreatment with NAC was successful in keeping the GSSG level low in comparison to the fish that received arsenic treatment alone. Furthermore, the activity of the enzyme glutathione reductase (GR), important in keeping the GSSG:GSH ratio low by reducing the GSSG back into GSH during oxidative stress, was found to remain inhibited in the initial days of exposure at both doses

(days 1–2) in arsenic-treated *C. batrachus* (Bhattacharya 2004). The GR activity recovered to the normal level on the third day of exposure in the case of arsenic-treated fish, whereas it remained at the basal level in NAC-preinjected fish throughout the experimental period. It could be concluded (Bhattacharya and Bhattacharya 2005) that arsenic treatment induced oxidative stress through the mediation of H_2O_2. It was found that the tissue H_2O_2 level in *C. batrachus* remained high in general. The preinjection with NAC was found to reduce such increases in tissue H_2O_2 level but still maintained it at a moderately higher level than the control.

Glutathione peroxidase (GSH-Px)

The GPx enzyme family consists of selenoproteins with both cytosolic and mitochondrial localization. Mitochondrial GPx are thought to play a major role in detoxification of H_2O_2 produced upon superoxide dismutation by mitochondrial SODs. The ability of GPx to reduce hydrogen peroxide and a large number of organic hydroperoxides enables it to protect against oxidative damage of biomembranes and macromolecules.

The activity of GPx in fish varies greatly among species. Rainbow trout and brook trout (*Salvelinus fontinalis*) have lower GPx activities than bluegill sun-fish (*Lepomis macrochirus*) and much lower activities than carp. Among cyprinids the highest activity is found in carp, with lower values for grass carp (*Ctenopharyngodon idella*), silver carp (*Hypophthalmichthys molitrix*), barbel (*Barbus barbus*) and crucian carp (*Carassius carassius*). Therefore, it has been suggested that GPx activities reflect feeding behavior of the fish, being highest in the omnivorous carp (Radi et al. 1985).

Environmental pollutants may induce GPx activity in fish but opposing results have also been found in many experiments. Activity of GPx increased, together with glutathione reductase, in rainbow trout after injection of tetrachlorobiphenyl (Otto and Moon 1995) and in carp after exposure to copper, but not to zinc sulfate. Sarkar (1997) reported an increase in GPx activity in *A. testudineus* exposed to acute higher doses of the metals Hg and Cd, and the pesticides metacid-50 and carbaryl, whereas chronic exposures did not affect the activity. Therefore, protection by GPx against ROS in fish may be questionable but fish, being more susceptible to oxidative damage, generally have higher GSH-Px activities (Hasspieler et al. 1994).

Glutathione reductase (GR) and the maintenance of redox ratio

The normal ratio of reduced to oxidized glutathione in cells is maintained by glutathione reductase enzymes which catalyze the reaction:

$$GSSG + NADPH + H^+ \rightarrow 2GSH + NADP^+$$

Very few reports are available on glutathione reductase activity and its role in various toxicant exposures. However, bearing in mind both in vivo and in vitro reports of varying responses of GSH and GPx in the case of exposure to different toxicants in fish, it seems obvious that GR activity must also be affected and would be indicative of generation of any oxidative stress. Styblo et al. (1995; 1997) demonstrated that arsenicals and arsenothiols, especially methylated trivalents, are potent inhibitors of GSSG reduction by GSH reductase. The inhibition constants for di (γ-glutamyl cysteinyl glycinyl) methyldithioarsonite and di-cysteinyl-methyl-dithioarsenite, the two possible intermediates of arsenic metabolism in vivo, were 0.009 and 0.018 mM, respectively (Styblo et al. 1995, 1997). Both the trivalent organo-arsenicals generated during biomethylation of arsenic and the arsenothiols formed in the cell by reaction with GSH could alter the cellular GSH:GSSG ratio by inhibiting GSH reductase.

In an interesting study with *C. batrachus* (Bhattacharya and Bhattacharya 2005) excess H_2O_2 was found to accumulate in the tissues during arsenic exposure. It was further observed that SOD was stimulated significantly in comparison to the control throughout the treatment period and both GPx and SOD activities were depressed in response to NAC preinjection. It was also found that arsenic treatment stimulated GPx, but its daily percentage stimulation profile was almost similar to that of SOD.

Imbalance in H_2O_2 metabolism in the peroxisome

It is known that peroxisomal acyl co-A oxidase (ACOX) participates in β-oxidation of very long-chain

fatty acids (LCFAs) and produce H_2O_2, while peroxisomal catalase could remove this H_2O_2 to keep its level low. It was also reported that ACOX and catalase activity increased in fish treated with arsenic. Interestingly, NAC pretreatment was also found to restrain the increase in peroxisomal ACOX activity in response to arsenic. However, the increase in ACOX activity was still found to be greater in comparison to the increase in catalase activity. It was thus proved that the imbalance in the peroxisomal peroxide-generating versus peroxide-degrading enzymes was not totally reversed by pretreatment with NAC, although it was significantly reduced. The time-dependent profile of total tissue catalase activity and peroxisomal catalase activity in response to arsenic was found to be similar, though there was a quantitative difference between them, indicating the presence of catalase in the cytosol. Therefore, the NAC pretreatment could prevent the inhibitory effect of arsenic treatment on the expression of this protein, but it could not increase the expression significantly in comparison to the control. However, for the cytosolic samples, Western blot analysis revealed that neither did the treated samples undergo downregulation of the expression of this catalase-like protein nor did NAC pretreatment increase its expression (Bhattacharya and Bhattacharya 2005).

Induction of stress proteins by arsenic

Metallothionein

Metallothioneins (MT) are a group of cytoplasmic proteins involved in metal regulation (Roesijadi 1992). They are a class of low-molecular-weight cysteine-rich metal-binding proteins found in a large number of prokaryotes and eukaryotes (Kägi and Kojima 1987). MT is a small protein easily induced by heavy metals, hormones, acute stress, and a variety of chemicals (Kägi 1993). Arsenicals are effective inducers of MT in mice (Kreppel et al. 1993a,b) and rats (Albores et al. 1992). Induction of MT by arsenicals appears to be regulated at the transcription level, because As^{3+} and As^{5+} increased the MT-I and MT-II mRNA levels, As^{3+} being more effective (Albores et al. 1992; Kreppel et al. 1993a, b). The potency of arsenicals to induce MT [As^{3+} > As^{5+} > MMA (monomethyl arsenic acid) > DMA (dimethylarsinic acid)] parallels the toxicity of arsenicals (Klaassen 1995). MT induction has been proposed as one of the adaptive mechanisms for tolerance to arsenic toxicity (Cherian 1995). In fish, the organs in which metallothioneins have been found in high concentrations are the liver, kidney, gill, and intestine (Roesijadi 1992). MT has been found at a very high concentration in the liver of mature animals after metal exposure (Chatterjee and Bhattacharya 1986). MT may constitute a defense mechanism that protects the cell from the reactive intermediates. Kägi and Schaffer (1988) suggested that this property is due to its high cysteine content, which act as neutralizing nucleophilic equivalents. The most striking feature of MT is that it not only binds with heavy metals, but its synthesis is also induced by Hg, Cd, Zn, and As (Agarwal and Bhattacharya 1990; Mukhopadhyay et al. 1994; Kuroshima 1995; Das et al. 1998). Liver appeared to be the most sensitive organ for increased MT synthesis following exposure to a number of metals. For goldfish (*C. auratus*) it has been proposed that hepatic MT protects the animal from metal xenobiotics by sequestration and detoxication (Suzuki et al. 1987) while Carpene et al. (1987) suggested that MT might interact with other metal enzymes during detoxication in fish.

There is only a lone report on arsenic-induced changes in the MT profile in fish (Roy and Bhattacharya 2005). Interestingly, the hepatic MT content expressed in terms of n mole ^{203}Hg incorporated g tissue^{-1} (71.44–72.69) in control *C. punctatus* was found to be higher than the renal content (67.21–67.88). On treatment with arsenic at first a significant decrease in hepatic MT was effected on days 1 and 2. In the later phases of arsenic treatment, the level of MT is highly enhanced, suggestive of induced synthesis of this stress protein. In the case of renal metallothionein two different patterns were observed in the two treatments. In 1/20 LC_{50}-treated fish the MT content did not cross the basal level, rather it remained significantly depressed on day 2 and 7, while in 1/10 LC_{50} treatment the MT level showed a biphasic response (Roy 2004; Roy and Bhattacharya 2005).

Induction of Hsp 70

Heat-shock proteins (Hsps) are a family of highly conserved proteins that play an important role in the

functioning of unstressed and stressed cells (Parsell and Lindquist 1993). Hsps are families of proteins that, when expressed, play an important role in the protection and maintenance of many vital cellular functions. It was found that Hsps offer resistance toward other events like hypoxia, ischemia, inflammation and the exposure of cellular toxins such as heavy metals, endotoxins, reactive oxygen species, oxidants, amino acid analogues, etc. (Atkinson and Walden 1985; Luc et al. 2001). Increased resistance toward stressful events has been described in a great variety of organisms, organs, and tissues. As the members of the HSP70 family are often prominent proteins expressed following environmental insults they are categorized under the group of stress proteins. These stress proteins are therefore very useful biomarkers that have been used to monitor the impact of environmental factors on various animal species, including many invertebrates (De Pomerai 1996; Eckwert and Köhler 1997; Lewis et al. 1999).

In a unique study on *C. punctatus* Roy and Bhattacharya (2005) reported that arsenic also induces the synthesis of heat shock protein 70 (hsp 70) in the liver and kidney. The day-wise profile showed that 1/20 LC_{50} (3.8 mg^{-1}) As_2O_3 induced maximum levels of hsp 70 on day 1. Again, although the level of expression tended to decline on all the remaining days of the experimental period, it always remained higher than that of the control. Similar temporal profiles of hsp 70 expression could also be seen at the 1/10 LC_{50} (7.6 mgl^{-1}) dose. High levels of hsp 70 were observed on day 1 in the liver, which however increased marginally on day 2. When the exposure period was extended, on day 7 the overall expression of hsp 70 appeared to have reduced, though it still remained higher than that of the control. On day 14 there was an augmentation in the level of expression of hsp 70. In the renal samples the expression of hsp 70, however, did not show any remarkable difference between the control and the treated samples (Roy and Bhattacharya 2005).

ROS-mediated effects on intracellular signaling and MAPK pathway

The multitude of chemical reactions involving ROS can target various functional groups on proteins, lipids or DNA and recent evidence points to involvement of O_2^- and H_2O_2 in oxidative modification of key signaling proteins (Ullrich and Bachschmid 2000). Under severe oxidative stress, damage to the cell could be nonspecific and lead to necrotic cell death leading to pathological conditions. When redox reactions play a role in cell signaling mechanisms, they target a restricted set of functional groups on specific protein targets, and these modifications are generally reversible by intracellular antioxidant mechanisms. Protein cysteine residues are common targets of oxidants. Thiol groups (–SH) can be oxidized to sulfenic (–SOH), sulfinic ($–SO_2H$) or sulfonic ($–SO_3H$) derivatives. Two thiol groups can also build a disulfide bridge (–S–S–) upon oxidation or form a mixed disulfide with glutathione (GSH) and destroy/activate protein function. Oxidation of the active site cysteine residue of protein tyrosine phosphatases by ROS and the formation of sulfenic acid derivatives inhibit the enzymes and result in the imbalance of phosphorylaiton/dephosphorylation by tyrosine kinases (Denu and Tanner 1998; Barrett et al. 1999). As protein tyrosine phosphatases can dephosphorylate MAPK proteins, inhibition of tyrosine phosphorylation can, in turn, activate downstream signaling cascades leading to the activation of the Ras-ERK (extracellular signal regulated kinase) pathways and c-Jun N-terminal kinase (JNK) and p38 MAPK kinase cascades (Qian et al. 2003).

Conclusions and perspectives

The toxic effects on fish of nonlethal concentrations of arsenic have been summarized herein. It is abundantly clear that arsenic induces an early response in fish. Arsenic is a potent inducer of hsp 70 and other stress proteins in fish and also reveals a strong interrelationship with stress signaling, cell death, and synthesis of stress proteins. A common biochemical phenomenon is involved in arsenic toxicity under both in vivo and in vitro exposure conditions, as observed in mammalian models at comparable exposure dosages and time scales (Yeh et al. 2002).

Several of the measured variables could be proposed as biomarkers of oxidative stress and effects. The central mediator of this oxidative stress appears to be H_2O_2, and increased activity of

peroxisomal ACOX in consonance with significant inhibition of peroxisomal catalase could be a potential source of excess H_2O_2 generation. Such basic information will be of immense help in assessing the impact of arsenic toxicity on freshwater fish as well as in developing a sensitive biomarker of aquatic pollution caused by the excessive release of arsenic into the freshwater. The MAP kinases are also likely to be involved in the final transduction of the xenobiotic signal in fish.

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