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Histopathological responses of newly hatched larvae of whitefish (*Coregonus lavaretus* s.l.) to UV-B induced toxicity of retene

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Abstract

Positively phototactic fish larvae may be exposed to increased ultraviolet-B (UV-B) radiation alone or, potentially and in addition, to polycyclic aromatic hydrocarbons (PAHs) such as retene (7-isopropyl-1-methylphenanthrene) at the egg or larval stages. Suspended and sedimental particulate material near pulp and paper mills may act as sources of retene in chemically contaminated lake areas. In laboratory conditions whitefish larvae were pre-exposed to retene (10, 32 and 100 μg/l), with relevant controls, and irradiated in semi-static tests for 3 h once a day (2 consecutive days) with two UV-B doses (CIE-weighted 2.8 or 5.4 kJ per m^2 per day) or with visible light only. These UV-B doses correspond with slightly subambient and 80% increases relative to the natural maximum daily doses of the solar UV-B in Finland in early May. The UV-B radiation alone increased mortality only slightly (2.7 and 4.0%, respectively). Similarly, no mortality was observed due to retene alone. On the contrary, simultaneous UV-B and retene exposure caused very high mortality to whitefish and all larvae died in the highest retene concentration. The photoinduced acute LC_{50} for retene was 13.3 μg/l. Retene treated fish exhibited signs of behavioral irritation and hypoxia during and after the exposure to UV light. Severe skin damages were detected in larvae exposed simultaneously to retene and UV-B. The structural signs of sunburn could also be seen in UV-B and solvent controls (DMSO) with UV-B. Even at the lowest retene concentration, the number of mucous cells increased significantly in simultaneous chemical and UV-B treatment. We consider the tissue reaction as protective response against UV induced retene toxicity. Further, regarding liver parenchyma, fish exposed to retene with UV-B had lesions, revealing hepatotoxicity. We suggest that synergism of the UV-B radiation and the photoactivating pollutants such as retene is a potential risk factor to be taken into consideration in lake areas chemically contaminated by the pulp and paper industry.

Keywords: Phototoxicity; Retene; UV-B; Histopathology; Skin; Liver

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1. Introduction

Ultraviolet-B radiation (UV-B, 280–315 nm) reaching the earth's surface has increased due to stratospheric ozone depletion caused anthropogenically (e.g. Stolarski et al., 1992; Kerr and McElroy, 1993; Taalas et al., 2000). In lakes, attenuation depths for ultraviolet radiation may range from a few centimeters in waters having high concentration of dissolved organic carbon (DOC) to over 10 m or more in some of the lowest DOC lakes (Kirk, 1994; Williamson et al., 1996; Huovinen et al., 2000). Many studies have demonstrated that enhanced UV-B radiation may be a threat to many organisms, also in aquatic ecosystems (e.g. Holm-Hansen et al., 1993). It can be lethal to early life stages of fish species and evoke multiple sublethal effects on planktonic organisms (Hunter et al., 1979, 1981; Blazer et al., 1997; Beland et al., 1999). On the other hand, whitefish larvae were very UV-B tolerant, probably due to melanin induction protecting them (Häkkinen et al., 2002).

Furthermore, UV-B can have harmful indirect effects such as the enhanced toxicity of environmental contaminants or synergism with pathogens (Holm-Hansen et al., 1993; Kiesecker and Blaustein, 1995). Many polycyclic aromatic hydrocarbons (PAHs), being relatively non-toxic as such, however, reveal enhanced toxicity because of UV-induced structural changes of the chemical (photomodification) or through photosensitization, the mechanisms caused by activation of chemicals bioaccumulated in tissues (Ankley et al., 1994; Mallakin et al., 1999; Little et al., 2000). Photoinduced toxicity of individual PAH-compounds (e.g. fluoranthene, anthracene, pyrene) has been documented e.g. in plants (Huang et al., 1995; Mallakin et al., 1999), zooplankton (Wernesson and Dave, 1997; Nikkilä et al., 1999), crustaceans (Boese et al., 1997), amphibians (Hatch and Burton, 1998; Monson et al., 1999) and fish (Oris and Giesy, 1985; McCloskey and Oris, 1991, 1993; Little et al., 2000).

In natural waters, retene (7-isopropyl-1-methylphenanthrene) is mainly formed anaerobically from resin acids, oleoresinous constituent in coniferous trees. It has been found in sedimenting particles (highest observed concentration 54 μg/g d.w.) contaminated by treated pulp and paper mill effluents as well as in the sediment surface up to 500–1600 μg/g in lake areas contaminated by the industry (Leppänen and Oikari, 1999; Leppänen et al., 2000). Although retene is hydrophobic by its nature, it is bioavailable to fish, presumably via desorption from sediments (Oikari et al., 2002). Besides aquatic systems, retene is formed thermally during forest fires and after burning soil may contain over 4 μg retene per g d.w. (Gabos et al., 2001). Municipal incinerators and oil refineries also have some retene in their effluents (Besombes et al., 2001).

In laboratory exposures, retene appears to accumulate in liver and muscle of fish (Brumley et al., 1997). Chronic exposures have shown that retene is teratogenic to fish embryos at rather low water concentrations ($\sim$32 μg/l or below) and induces the mixed function oxygenase (MFO) system in fishes (Fragoso et al., 1998; Billiard et al., 1999). Further, in a previous study, retene was phototoxic to *Daphnia magna* at concentrations revealing no toxicity without UV-B (Huovinen et al., 2001).

Lake whitefish (*Coregonus lavaretus* s.l.) spawn in late autumn and lay their eggs on the lake bottom, where they may come in contact with possible xenobiotics. In spring, newly hatched larvae aggregate in water layers near the surface or shallow littoral areas (Karjalainen et al., 1998). In May, positively phototactic larvae (Shkorbatov 1966; Karjalainen et al., 1998) are potentially exposed to solar UV-B and, simultaneously, to retene bioavailable from water or particulates (Leppänen et al., 2000; Oikari et al., 2002). The objective of this study was to find out if retene evokes lethal phototoxicity in newly hatched larvae of whitefish and, further, if it causes sublethal toxicity as evaluated by histological endpoints. Liver, which accumulates retene (Brumley et al., 1997), is a potential target organ due to its large blood supply and great metabolic capacity (Hinton et al., 2001). On the other hand, skin can also be a target organ in aqueous exposures of larval fish utilizing skin respiration during development. In bottom-dwelling species the skin may even have direct contact with

contaminated sediments and their pore waters (McKim and Lien, 2001).

2. Materials and methods

2.1. Organisms

1-day-old larvae of whitefish were used as test animals. Before hatching, the fertilized eggs were incubated at 4 °C in cylindrical glass funnels with upstream water flow. Once hatched at 6 °C, larvae were transferred to rearing aquaria and divided randomly for different treatments, also conducted at 6 °C.

2.2. Chemicals

Retene (7-isopropyl-1-methylphenanthrene, purity 98%) was obtained from ICN Biomedicals (Costa Mesa, CA, USA). A stock solution (100 μg/ml) was made in dimethylsulfoxide (DMSO) used as a carrier. The concentration of DMSO in the solvent controls was the same as that of the highest retene concentrations, never exceeding 0.1%.

2.3. Light regimes

Phototoxic responses are dependent on UV absorption characteristics, i.e. compounds that absorb relatively low amounts of UV radiation have low phototoxicity. Retene has an absorption spectrum peak in the UV-B region, whereas, for instance anthracene and pyrene in the UV-A region. It follows, that the phototoxicity of retene can be related to UV-B radiation (Huovinen et al., 2001).

Two adjustable Q-Panel UVB-313 lamps (Q-panel laboratory products, Bolton, UK) were used as sources of UV-B (280–315 nm). Deleterious UV-C (under 280 nm) radiation was blocked with a cellulose diacetate filter (Clarifoil), which was replaced after each 3-h exposure occasion. In control treatments, only visible light (Philips TLD 36 W/950 daylight) was present. UV-B irradiation in different treatments was measured by a Hamamatsu Photonic Multichannel Spectral analyzer (Model PMA-11), integrating the wavelength area from 280 to 380 nm. The UV-B doses were calculated as J/m^2 (CIE-weighted), i.e. the action spectrum used in experiments was specific for human erythema (McKinlay and Diffey, 1987). However, it includes biologically most harmful shorter wavelengths that earth receives and can be used as an alternative model for biological effects to all organisms.

2.4. Experimental procedures

At the beginning, whitefish larvae were pre-exposed to retene (nominal concentrations 10, 32 and 100 μg/l) or controls (water and DMSO, used as a carrier, in aerated water) in Pyrex glass bowls (volume 1 l). After a 24-h accumulation period, larvae were further exposed to retene or to controls (test solutions were renewed daily before UV-B irradiation by carefully replacing 80% of each solutions) and, in addition, irradiated once a day (altogether two times) with either UV-B (2.8 or 5.4 kJ/m^2) or visible light for 3 h (between the 12:00 and 15:00 h) to simulate midday exposure. For comparison, according to measurements of the Finnish Meteorological Institute in Jokioinen (60.82 N, 23.50 E), the maximum erythemal (CIE) weighted daily dose was approximately 3 kJ/m^2 in May, 2000. Thus, respectively, the doses applied were slightly subambient (7% under) and 80% higher than the seasonally adequate natural maximum. All treatments and controls were replicated three times, each comprised of 25 newly hatched (age less than 12 h) whitefish larvae. Photoperiod (visible light) in the experiment room was 16:00-h light:08:00-h dark cycle. Water temperature was monitored twice a day, staying similar in the different treatments. Behavioral responses were monitored four times a day, also during irradiation. Dead larvae were removed and counted daily. After 2 days the larvae were anaesthetized with MS222 (50 mg/l) and immediately sampled for histological analyses.

2.5. Histology

Only fish exposed to the higher UV-B level (5.4 kJ/m^2) were used. Randomly sampled larvae (10 per treatment) were analyzed for histology,

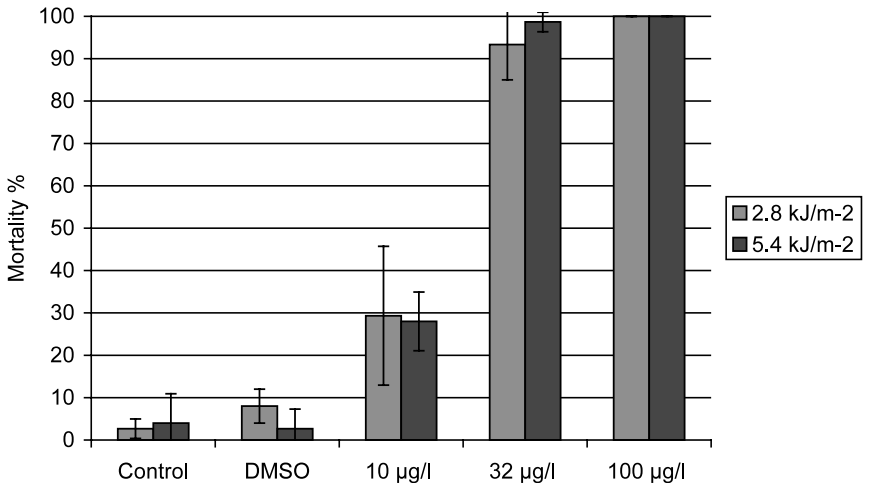

Fig. 1. Increased mortality of newly hatched whitefish (*C. lavaretus* s.l.) caused by UV-B irradiation induced phototoxicity of retene. Larvae were pre-exposed to retene (nominal concentrations 10, 32 and 100 µg/l) or controls. After a 24-h accumulation period, larvae were irradiated once a day (altogether two times) with either UV-B (2.8 or 5.4 kJ/m^2) or visible light for 3 h. Bar denotes standard deviation. There was no mortality without UV-B radiation.

although in the 32 µg retene per l treatment with UV-B, all nonmoribund specimens (four out of five) were examined. Larvae were fixed for 24 h in 10% buffered formalin. Once fixed, the samples were dehydrated through a graded series of ethanol solutions up to 100%, followed by xylene prior to embedding in paraffin. Animals were sectioned longitudinally along the vertical axis using a Leica microtome at 5 µm. Haematoxylin and eosin (HE) stained sections were prepared from each tissue block and examined at $\times 400$ and $\times 650$ magnifications using an Olympus IX70 stereomicroscope. Histological abnormalities detected in liver and skin were recorded. Lesions and tissue alterations were defined using several slides from each fish. The severity of skin lesions was classified to four groups depending on the percentage of the dorsal skin that had lesions: (0) healthy skin, (1) minor lesion ($\leq 1\%$), (2) pronounced (1–5%) and (3) severe skin lesion ($\geq 5\%$). The postcranial dorsal sector of longitudinal sections in larvae was selected for examination of dorsal skin lesions and counted for the number of mucous cells. Selected sections were, besides H&E, also stained with Periodic Acid Schiff (PAS)–Alcian blue–Mayer's haematoxylin (Mowry, 1968).

2.6. Statistical analysis

Probit-analysis was used to calculate LC_{50}-values for the different UV and retene combinations. Effects of different UV-B or/and retene treatments on the numbers of mucous cells per mm skin of the whitefish larvae were tested by the one-way ANOVA and Tukey's test.

3. Results

3.1. Mortality

No mortality was observed among controls or vehicle controls and all larvae survived in retene treatments without UV-B radiation. At both UV-B radiation levels applied (2.8 and 5.4 kJ/m^2 per day) mortality was low (2.7 and 4.0%, respectively). On the contrary, UV-B radiation induced toxicity of retene significantly (Fig. 1). When exposed to the highest retene concentration (100 µg/l), together with UV-B, all animals died. Further, only five individuals (6.6%) survived in the 32 µg/l treatment. After the first irradiation period slight mortality (2.6%) was observed only in the highest retene treatment (100 µg/l) with UV-B

(5.4 kJ/m^2), but 6–8 h after the second exposure period mortality was dramatic. The calculated EC_{50}-values for retene was 13.3 μg/l (96% confidence level) with both irradiation levels included (12.7 μg/l in high and 13.6 μg/l in low UV-B dose level, respectively, with no statistical difference).

3.2. Behavioral responses

Retene treated fish exhibited behavioral changes during and after exposure to UV-B. Initial symptoms were observed during the first irradiation and immediately after. Hypoxia was suggested by a tendency to swim to the water–air interface. Later, fish expressed uncontrolled spiral swimming and had tremors in the cranial area. Most severely affected fish remained at the bottom of the bowl. Practically all larvae had these symptoms in the highest retene concentration (100 μg/l), as did most fish in the two other retene concentrations. After the first UV-B exposure, however, the response was reversible and most larvae recovered within 5–6 h. After the second irradiation, these symptoms recurred, but this time they were irreversible and the larvae died. Larvae exposed to retene or UV-B alone had no behavioral symptoms.

3.3. Skin and liver

Lesions were observed only in UV-B treatments and, most distinctly, due to simultaneous UV-B and retene exposure (Fig. 2). Skin of the healthy larvae was uniform and consisted of epidermis of one or two cell layers, lying over the melanophores and dorsal muscle (Fig. 3A). The skin of the newly hatched whitefish was partly undifferentiated without distinct dermis zone. Whitefish at this developmental stage have no scales in the skin.

At a retene concentration of 32 μg/l with simultaneous UV-B exposure (5.4 kJ/m^2), the cell membrane integrity of the skin was lost and large areas of the epidermis consisted of necrotic cells having hyperchromatic and shrunken nuclei. Severely affected cells appeared as foci of dense nuclear droplets or granules, characteristic of the classic sunburn response (Fig. 3B and C; Bullock and Coutts, 1985; Noceda et al., 1997). Also sloughing and vacuolization, resulting in uplifting,

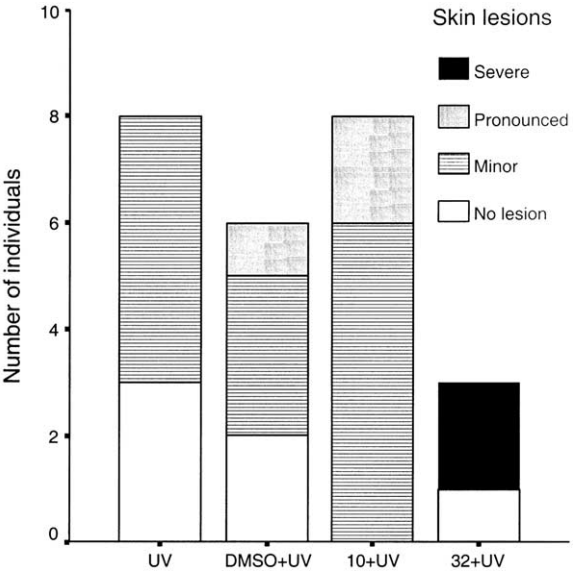

Fig. 2. The number and severity of skin lesions observed in different UV-B plus retene treatments. The severity of skin lesions was classified to four groups, depending on how large percentage of the dorsal skin was affected (see Section 2).

was observed in the epidermis (Fig. 3B and C). Same kinds of lesions were also observed in treatment 10 μg retene per l with UV-B, but changes were less dramatic. Most observed lesions evoked by retene plus UV-B were in or near dorsal and caudal fins (Fig. 4).

Additionally, minor changes were seen in the skin of larvae that were exposed either solely to UV-B irradiation or DMSO with UV-B, representing a reference situation without phototoxic retene. In these animals the integrity of the skin was not lost, but the epidermis had a few cells with nuclear droplets or necrotic nuclei in it. The retene concentration alone had no observable effect on larval skin.

In newly hatched whitefish, differentiation of mucous (goblet) cells can be observed, and their numbers were counted from histological sections targeted to the dorsal side of animals. Mucous cells were PAS-positive and with alcian-blue PAS method they appeared strongly stained (bright blue through microscope; Fig. 5). There was a statistically significant difference in numbers of mucous cells in the dorsal skin ($P < 0.001$, $F = 3.418$, one-way ANOVA). Fish that were exposed

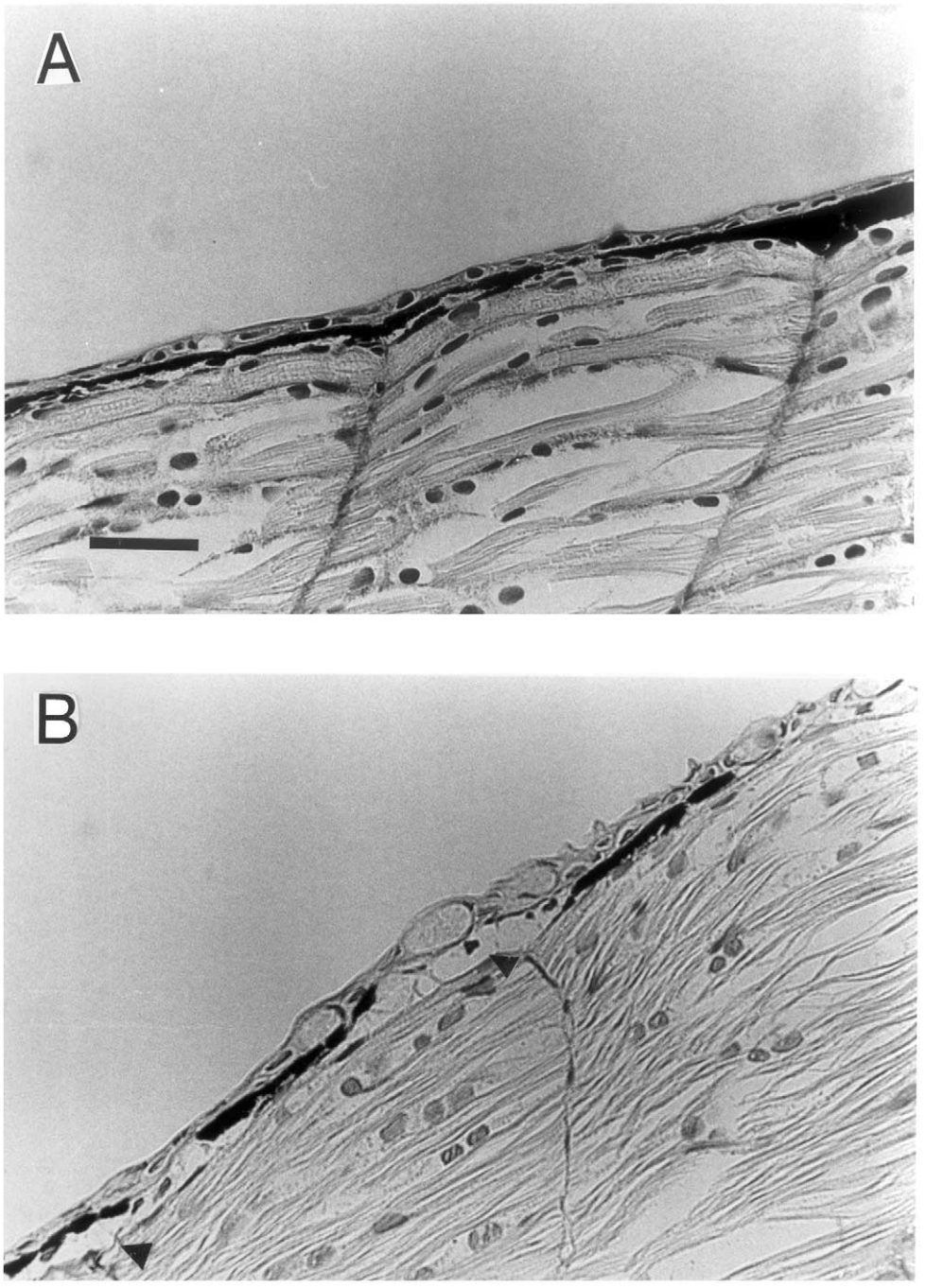

Fig. 3. (A) Skin of a healthy (control) newly hatched whitefish larva, with uniform epidermis of one or two cell layers, laying over the melanin and muscle. (B) Skin of a larva simultaneously exposed to UV-B (5.4 kJ/m^2) and retene (32 μg/l). Several mucous cells open to the surface and vacuolization can be seen under the epidermis, uplifting the skin (indicated by arrowheads). (C) Severe lesions in the skin of a larva exposed to retene (32 μg/l) and UV-B. Part of the skin was dead and nucleal granules (arrowhead) can be seen (HE, scale bar = 10 μm).

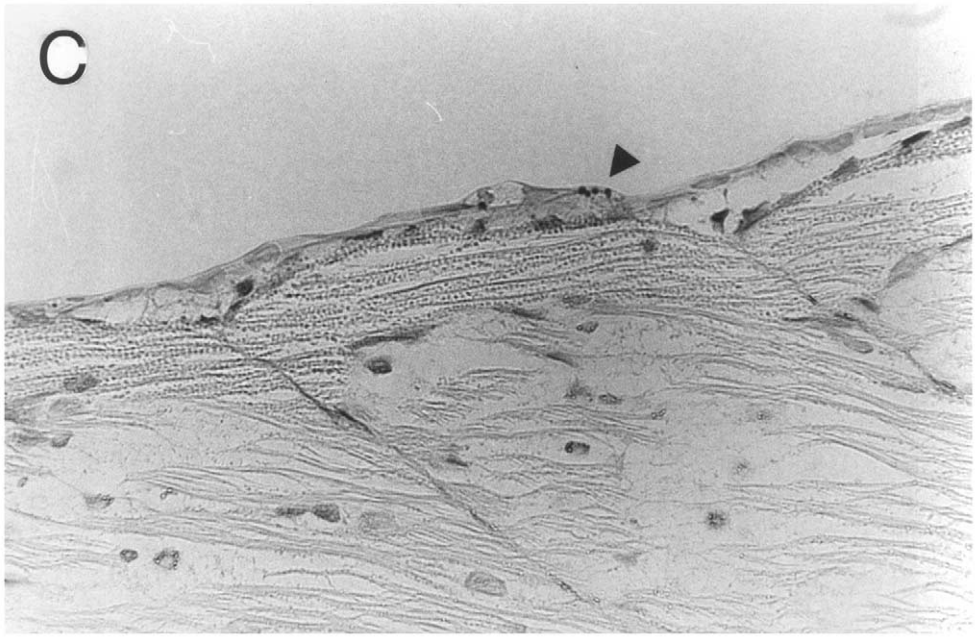

Fig. 3 (*Continued*)

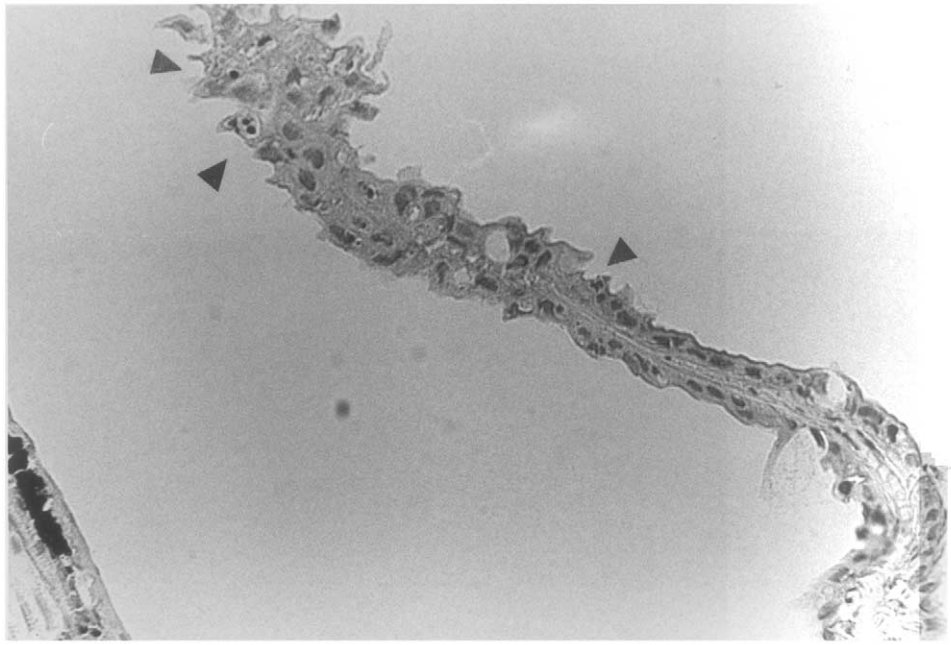

Fig. 4. Granular nuclei (indicated by arrowheads) in the dorsal fin of a whitefish larva treated by 10 μg/l retene with UV-B (HE, scale bar = 10 μm).

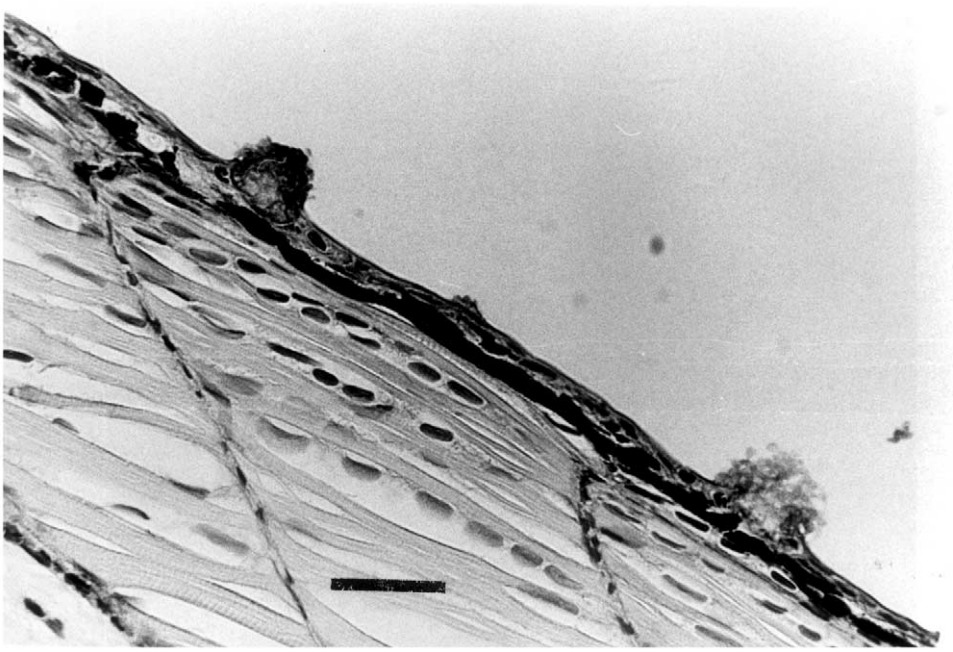

Fig. 5. Two mucous cells opening to the skin surface of a UV-B exposed larva. No lesions can be observed. With alcian-blue PAS method substance inside of mucous cells stained bright blue (scale bar = 10 μm).

Table 1
Average (±S.D.) number of the mucous cells per mm in dorsal skin of the newly hatched whitefish

Treatment	Number of animals	Mucous cells per mm skin
Control	10	4.03±0.89
DMSO	7	4.10±1.56
10 μg/l	7	3.95±0.79
32 μg/l	8	4.39±1.58
100 μg/l	8	5.32±1.24
UV alone	8	4.81±0.93
UV and DMSO	5	4.84±0.97
UV and 10 μg/l	8	5.85±0.82*
UV and 32 μg/l	3	6.62±1.41*

Asterik denotes statistical significance ($P < 0.05$) related to controls.

simultaneously to UV-B and retene (32 μg/l) had significantly more (64%) mucous cells in the dorsal skin than the control fish (Table 1; $P < 0.05$, Tukey's test). Similarly, UV-B plus retene at the lower concentration (10 μg/l) increased the number of these cells by 45% ($P < 0.05$, Tukey's test). Interestingly, larvae that were exposed to UV-B irradiation, with or without DMSO, or 100 μg/l retene alone had more (19, 20 and 32%, respectively) mucous cells than in respective controls, but the differences were statistically non-significant ($P > 0.05$).

Compared with control larvae, the highest retene concentration (100 μg/l) without UV-B radiation, had no effect on liver structure ($n = 10$): the normal architecture was maintained and hepatocytes contained normal spherical nuclei (Fig. 6A). The cytoplasm area of hepatocytes remained unstained in every occasion, because they were filled with lipid or glycogen deposits. Also in treatments 32 and 10 μg/l of retene, the fish livers were normal, as well as in control treatments (DMSO with or without UV-B and UV-B alone; $n = 10$ per treatment).

On the other hand, in treatment with retene plus UV-B, distinct histopathological changes were observed (Fig. 6B). Three out of four fish that were examined to 32 μg/l with UV had several hypercromatically stained, shrunken nuclei,

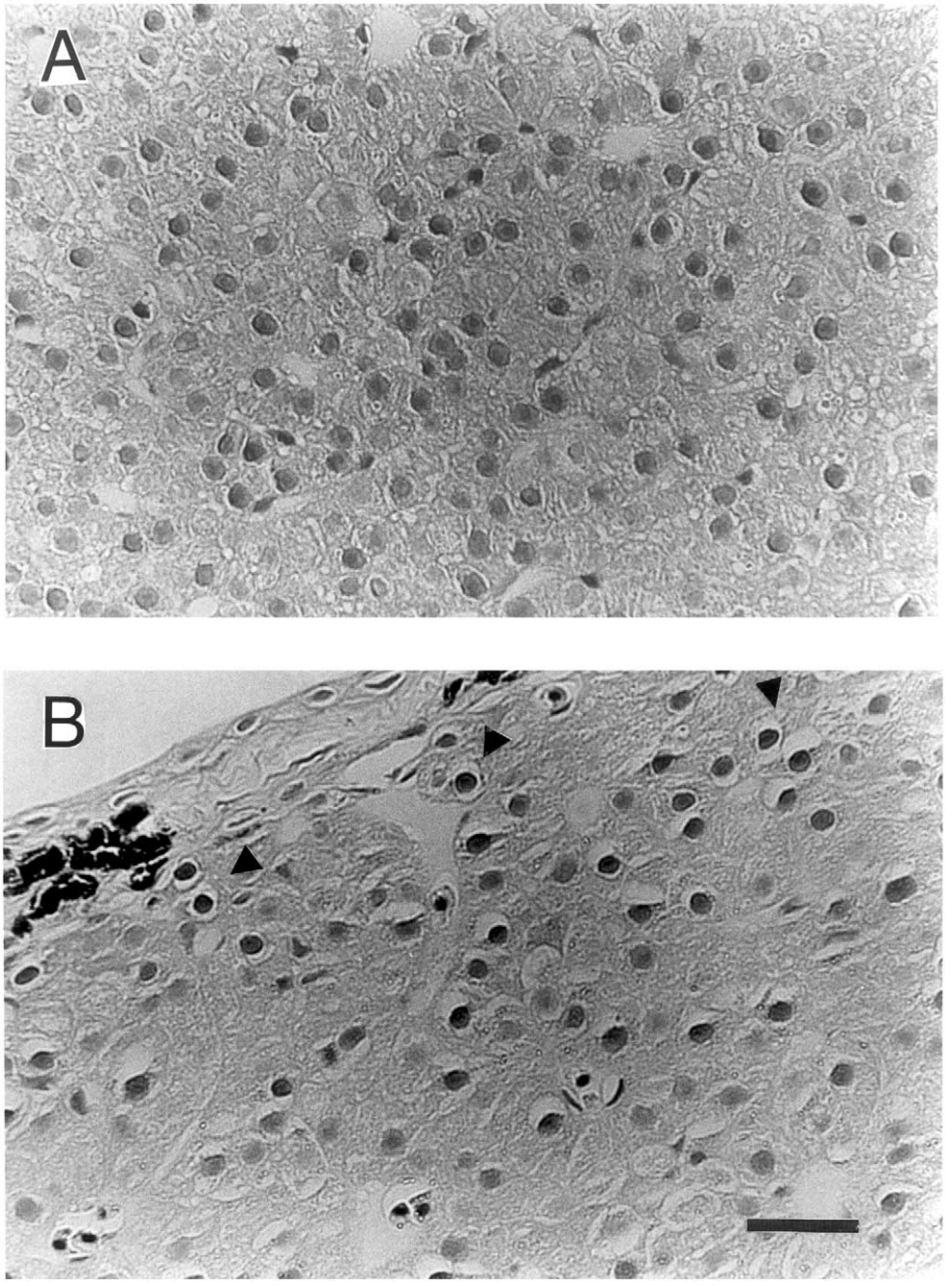

Fig. 6. (A) Structure of liver tissue in control larva with nuclei having normal spherical appearance. (B) The liver of a whitefish exposed to 32 μg/l retene and UV-B (5.4 kJ/m^2). Hepatocyte nuclei have a necrotic appearance, notably darkened and vacuolated perinuclear space, rounded with unstained area (indicated by arrowheads; bar scale = 10 μm).

rounded with unstained space, which appeared as foci of necrotic lesions (Fig. 6B). Also two out of four fish had some granulous nuclei in their liver. Unfortunately mortality of animals was very high

in that exposure level, decreasing the number of individuals available for sublethal histological analysis. Similar, but minor alterations were observed in six out of ten individuals at exposure level of 10 μg retene per l with UV-B.

4. Discussion

Many studies have documented increased photoinduced toxicity of individual PAH-compounds (e.g. fluoranthene, anthracene, pyrene) in many taxonomic groups (McCloskey and Oris, 1991; Huang et al., 1995; Boese et al., 1997; Hatch and Burton, 1998; Mallakin et al., 1999; Nikkilä et al., 1999; Little et al., 2000). Whereas, it is apparent that retene by itself was nonlethal to larval whitefish, its toxicity in the presence of UV-B was most dramatic, evidenced by acute lethality at low concentration (13.3 μg/l). The result substantiates the recent observation on acute toxicity of retene to *D. magna*, also revealing that retene is not as phototoxic as some other PAH-compounds, for example pyrene or anthracene (Huovinen et al., 2001). However, being important ecologically, no information currently exists on the minimum effective dose of UV-B, enhancing phototoxicity, for sensitive fish larvae potentially exposed to retene in polluted waters.

Previous studies have shown that UV-B radiation is detrimental to fish, especially at embryonic and larval stages. The most severe effects of UV-B radiation impair larval development and decrease offspring recruitment (e.g. Hunter et al., 1979; Kouwenberg et al., 1999; Browman et al., 2000). However, a recent study reveals that boreal species and populations of coregonids can be very UV-B tolerant and survive with UV-B daily doses up to 34% higher than seasonal average (Häkkinen et al., 2002). The results of this experiment strongly support the view.

Though direct impact of enhanced UV-B may be negligible to larval whitefish exposed in May, indirect chronic effects due to photoinduced toxicity may be deleterious. As in phototoxicity studies earlier (Oris and Giesy, 1985; McCloskey and Oris, 1991), retene caused signs of behavioral irritation and hypoxia during simultaneous UV-B exposure. Juvenile sunfish (*Lepomis macrochirus*) exposed to PAH and UV had increased ventilation rate and coughing (Oris and Giesy, 1985; McCloskey and Oris, 1991). Cellular damage (mainly in gills), caused by oxidative stress, has been suggested to cause PAH induced phototoxicity in fish (Oris and Giesy, 1985, 1987; McCloskey and Oris, 1993; Weinstein et al., 1997; Choi and Oris, 2000). Due to direct exposure to UV, skin can be the primary target organ in aqueous exposures of larval fish that utilize skin respiration during early development (McKim and Lien, 2001). Accordingly, our study showed structural phototoxicity of retene induced by simultaneous UV-B, causing the extensive and multiple skin damages in 3-day-old posthatch larvae of whitefish. As with earlier histological UV studies (Bullock and Coutts, 1985; Berghahn et al., 1993; Noceda et al., 1997), some of the observed lesions resembled classical sunburn response, appearing as foci of granular nuclei, sloughing and vacuolization of the skin of larvae. It is hypothesized, that these so called 'sunburn cells' may be apoptotic (Noceda et al., 1997), but so far there is no direct evidence supporting that. Minor sunburn damages were also seen in UV-B control treatments.

We also monitored changes in mucous cells, considered as protective structural elements in the skin against UV plus retene exposure. The suggested functional significance of fish epidermal mucus include osmoregulation, protection from abrasions, entanglement of particulate materials, defense against pathogens, UV radiation and parasites, reduction of swimming drag or friction, and protection against environmental contaminants (McKim and Lien, 2001). In our study the number of mucous cells increased significantly after simultaneous retene and UV treatments. Somewhat surprisingly, the result was opposite to previous studies indicating decreased density of mucous cells after exposure to UV-B (Little and Fabacher, 1994; Blazer et al., 1997; Noceda et al., 1997). One reason for the difference can be the higher tolerance of whitefish to UV radiation. Beneath the epidermis of larvae there were plenty of melanophores, suggesting another mechanism of protection against UV-radiation in whitefish. An evidence for acquired acclimation to enhanced

UV-B radiation by induced melanin production in whitefish larvae was indicated earlier (Häkkinen et al., 2002). However, in the retene concentrations 10–32 μg/l with additional UV-B, these protective mechanisms were insufficient.

Our results show that UV-B irradiated retene is also acutely histotoxic to the liver of young whitefish. The newly hatched larvae, exposed to 10 or 32 μg/l retene with UV-B, showed hepatocytes with necrotic nuclei. The mechanism of retene-induced phototoxicity in the whitefish liver, may be oxidative stress. In fact, Choi and Oris (2000) using fish liver microsomes, obtained evidence that lipid peroxidation is an important mechanism in PAH phototoxicity. Therefore, hepatotoxicity in larval whitefish is not surprising, because retene is known to accumulate in the liver of fish (Brumley et al., 1997). Further, the impact at an apparently remote site of action, the liver, is not necessarily unexpected due to possible accumulation and circulatory transfer of retene's photo-oxidation products originally formed in ambient water or in the skin. Fish skin observations suggest that UV effects on fish and its reactions to photoproduct, but toxic compound could be formed outside as well. In all, it is not clear if the hepatotoxic agent had been created outside or in the fish skin and transferred to liver.

In the present study whitefish larvae were exposed for 3 days to retene, of which 2 last days also to UV-B, accounting for about 40% of eleutheroembryonic phase (ca. 7 days at 6 °C). No signs of blue-sac disease (Billiard et al., 1999) were visible in our whitefish exposed for 3 days posthatch to retene, with or without UV-B. It is, therefore, expected that the sensitivity of whitefish larvae would further increase with longer-term combined exposures. In fact the characteristic effect of retene, without UV-B, to cause symptoms of blue-sac disease (yolk edema) was observable during the latter half of the period from hatch to complete absorption of yolk (Billiard et al., 1999).

We did not analyze whether photosensitization or photomodification was the mechanism of UV induced phototoxicity of retene. However, in studies with *D. magna*, accumulation of the retene in animal before UV exposure was essential for the induced toxicity. Further, no photoproducts, possibly formed in water, were acutely toxic without further exposure. Thus, the enhanced acute toxicity was primarily due to internal photosensitization reactions rather than photomodification (Huovinen et al., 2001). On the other hand, after irradiation of retene two new peaks were observed in the GC–MS analyses, even when exposed to a low UV-B dose (1 kJ/m^2; Häkkinen and Oikari, unpublished), suggesting the importance of ambient photomodification reactions.

A rationale behind this work was to assess added risks theoretically evoked to lake ecosystems by increased UV-B radiation and photoactivating chemicals in combination. However, more knowledge is needed on various possibilities in which retene may truly interact with UV-B. First, retene can end up to the water column via resuspension from sediment during the spring overturn or via bioturbation, as well as in current discharges from operating mills (Leppänen et al., 2000). Further, retene occurs in high concentrations in sediments contaminated by resin acids from pulp mill effluents (Leppänen et al., 2000) and it is bioavailable to benthic fishes and invertebrates (Nikkilä et al., 2001, Oikari et al., 2002). Thus, a potential risk appears that newly hatched whitefish larvae, which are very phototactic, get exposed to retene via its desorption from sediment and by surfacing become exposed UV-light and the phototoxicity of retene. The exposure route suggested can be relevant.

In conclusion, based on this laboratory study it is evident that retene with simultaneous UV-B radiation is highly toxic to newly hatched whitefish larvae. On the other hand, vertical UV-B conditions vary nonlinearly in different lakes. In humic lakes UV-B penetration can be only few centimeters, but in clearest lakes in Finland UV-B can penetrate deeper than 1 m (Huovinen et al., 2000). Several environmental factors, such as DOC, colored humic substances and light conditions may seasonally and episodically have strong influence to photoinduced toxicity of PAHs. DOC, besides reducing the bioaccumulation of PAHs to fish (Weinstein and Oris, 1999), may strongly reduce the penetration of UV-B radiation to the lake (Williamson et al., 1996; Huovinen et al., 2000).

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