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ORIGINAL PAPER

Effects of atorvastatin and its photoproducts on three trophic levels of the aquatic food web

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Abstract

Atorvastatin (ATV) photodegradation with radiation relevant to the shortest solar radiation wavelengths reaching the Earth's surface was studied. Fourteen products of atorvastatin photodegradation (P-ATV) were identified by HPLC-HRMS/MS, chemical structures were proposed for twelve of them. The suggested structures include decarboxylated, dehydrated ATV, ether-like structures and phenanthrene-like byproducts. Toxicity assays revealed that exposure to ATV had a greater negative effect on the growth of the green algae *Desmodesmus subspicatus* than the exposure to P-ATV (IC20 values 140 µg/l and 475 µg/l, resp.). The opposite trend was observed with *Daphnia magna* where P-ATV was 12 times more toxic than ATV with LC50 values of 3.25 mg/l and 38.5 mg/l, resp.). Long-term exposure to environmental concentrations showed that P-ATV significantly lowered the number of juveniles of *D. magna*, while ATV had no effect. Neither ATV nor P-ATV affected the interclutch period or the number of clutches. The higher toxicity of P-ATV was observed also in the 96-h test with *Danio rerio* embryos (the determined LC50 values for ATV and P-ATV 5.56 mg/l and 2.74 mg/l, resp.). Both groups of embryos of *D. rerio* (exposed to ATV or P-ATV) showed malformations such as heart and yolk sac oedema with the more pronounced effect in P-ATV. The novelty of the study is in a significant contribution to the understanding of the photochemical degradation of atorvastatin under UV light conditions relevant to surface waters together with the demonstration of deleterious effects of photoproduct mixture on the selected aquatic organisms of different trophic levels.

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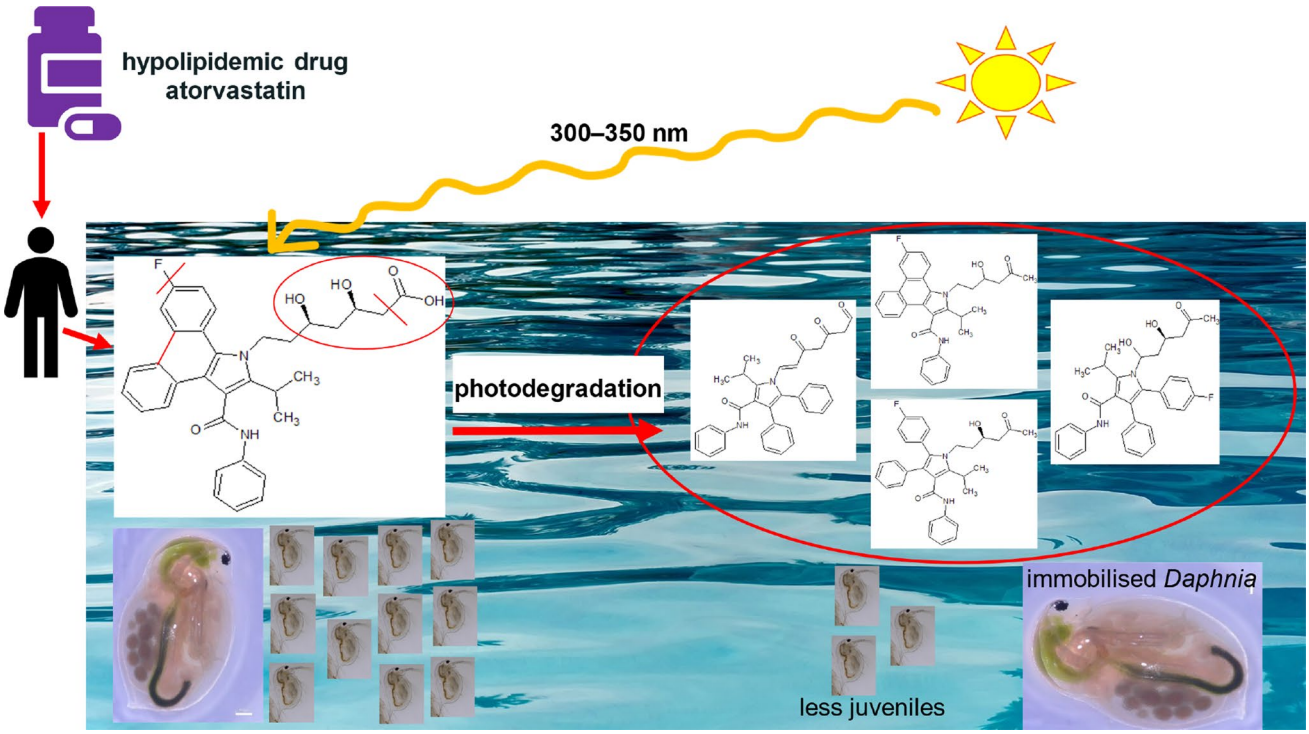
Graphical abstract



Keywords Atorvastatin · Photochemical degradation · HPLC-HRMS/MS · *Daphnia magna* · *Desmodesmus subspicatus* · *Danio rerio*

Introduction

Atorvastatin (ATV) is a member of the group of hypolipidemic drugs known as statins that lower the cholesterol level in the body by suppressing its biosynthesis (Goldstein & Brown 1990; Stancu & Sima 2001) via the competitive inhibition of a key enzyme (Lindsey & Siddique 2020). Atorvastatin belongs to the most prescribed pharmaceuticals worldwide—in European countries, it is in the top ten drugs (Strampelli et al. 2020), in the US it ranked position one in the number of prescriptions (ClinCalc 2024).

Most of the administered ATV (98%) is eliminated from the human body unchanged (Lennernäs H. 2003; Vítovec et al. 2017), which means that ATV enters municipal wastewater, and subsequently – since wastewater power plants are not designed to eliminate micropollutants – the surface waters. ATV has been repeatedly detected in surface waters worldwide in concentrations from ng/l to hundreds of µg/l (Conley et al. 2008; Lee et al. 2009; Kleywegt et al. 2019; Goswami et al. 2022).

Recently, adverse effects of micropollutants, including pharmaceuticals, on aquatic organisms have been the subject of investigation. Statins at environmental concentrations were found to interfere with lipid metabolism by blocking fatty acid β-oxidation (Zhao et al. 2024a) to alter the expression of some genes in fish, e.g. those involved in heart contractions and calcium ions binding (Ying Han et al. 2022) or genes related to membrane transport, biotransformation, and oxidative stress responses (Ellesat et al. 2012). The exposure also led to changes of biochemical parameters such as reduction in cholesterol, triglycerides, and sex hormones (Al-Habsi et al. 2016) and developmental parameters such as hatch rate, body length heart rate, and spontaneous movement as well as oxidative stress in embryos/larvae leading to abnormal developmental processes (Zhao et al. 2024b).

Nevertheless, not only fish but invertebrates such as mollusks (a bivalve), amphibians (an aquatic frog), and crustaceans (daphnia) were shown to be affected by statins. Exposure of a marine bivalve to ATV in concentration as low as 1 µg/l resulted had an impact on energy homeostasis – it led to a significant elevation of the basal metabolic rate thus depleting body energy reserves (Falfushynska et al. 2019). With an aquatic frog, alterations in cholesterol biosynthesis and gene expression patterns were detected (Johnson and Griffitt 2024). Exposure of daphnia was followed by a notable prolongation to the first brood and a

lower number of neonates were observed (Hu et al. 2022). Chronic exposure to ATV led to the accumulation of lipids in daphnia and adversely affected the growth and reproduction parameters (Wang et al. 2024).

According to Zhang et al. (2020), ATV belongs to the dominant drugs that may pose an environmental risk in contaminated water bodies since the effect concentration and the surface water concentration partially overlap.

Because surface waters are exposed to sun radiation, photochemical transformation pathways should be considered in connection with micropollutants. Several authors focused on the exploration of the photochemical behaviour of ATV in the aquatic environment (Lam et al. 2005; Razavi et al. 2011; Wang et al. 2018; Oprica et al. 2021) but there is still a lack of knowledge about the nature and adverse effects of the formed photoproducts. Montanaro et al. (2009) observed pyrrole ring oxidation and cyclisation to a phenanthrene derivative after exposure of ATV to sunlight. Ping et al. (2021) identified several oxidised photoproducts of ATV after exposure to UV light.

Ping et al. (2021) studied the toxicity of photoproducts of ATV degradation by UV light in connection with the removal of the drug from wastewater; the UV photolysis seemed to be effective for the drug removal, but they observed increased toxicity of photoproducts. Thus they concluded that the ecological risks of the process could not be ignored, since the process did not lead to complete mineralisation while producing products more toxic than the parental compound.

A study by authors Klementová et al. (2021) on the water plant *Lemna minor* demonstrated that even though ATV itself does not exhibit an observable effect measured as leaf area growth inhibition, exposure to the mixture of ATV photoproducts formed under irradiation with light relevant to surface waters led to a significant inhibition in leaf area growth.

This study has focused on the following goals:

- To get better insight into possible photochemical degradation of ATV under the conditions relevant to natural surface waters.
- To identify products of photochemical degradation by using high-resolution mass spectrometry and suggest the structure of the products.
- To perform toxicity assays on three aquatic organisms of different trophic levels (algae, daphnia, and fish embryos) and compare the effect of ATV itself with those of the photoproduct mixture.
- To use these results for evaluation of the pharmaceuticals' environmental impact considering their photochemical transformation in the aquatic environment

This work is novel first in the identification of atorvastatin photodegradation products that may be formed under environmentally relevant UV conditions and second in demonstrating the adverse effects of the photoproduct mixture on aquatic organisms across different trophic levels.

Materials and methods

Chemicals

ATV (in the form of calcium trihydrate, more than 99.9%, European Pharmacopeia Reference Standard, was purchased from Merck, Germany, its stock solution at a concentration of 50 mg/l was prepared by dissolution in double-deionised water (Smart2Pure6UV/UF, Thermo Scientific). $FeCl_3$ p.a., Lachema, Czech Republic) was used for experiments in the photocatalytic reaction.

Formic acid (LC–MS grade) for HPLC and UHPLC was purchased from Fisher, acetonitrile (LC–MS grade) from VWR.

Irradiation procedure

Irradiation of ATV with light simulating the shortest wavelengths of solar radiation reaching the Earth's surface was carried out in a Rayonet R100 reactor equipped with twelve RPR 3000 Å lamps emitting light at a wavelength range of 250 – 350 nm; to exclude wavelengths below 300 nm samples were irradiated in optical glass cuvettes covered with PTFE lids.

Actinometric measurement using ferrioxalate actinometry resulted in the value of 8.5×10^{-8} ein s^{-1} per the cuvette surface area.

For further experiments with irradiated samples, the P-ATV mixture was used.

Analyses of samples

HPLC analysis was used to determine the kinetic profiles of ATV photochemical degradation. The analysis was performed on HPLC (Thermo Scientific Dionex Ultimate system 3000 (USA), column Phenomenex Kinetex® 5 µm EVO C18, 30 × 2.1 mm, mobile phase water with 0.001% HCOOH and acetonitrile 0.6:0.4 (v/v) with PDA 3000RS spectrophotometric and FLD 3000RS fluorescence detectors).

TOC analysis was carried out to find whether the organic carbon is degraded to CO_2. The total organic carbon content of both non-irradiated and irradiated ATV samples was determined as nonpurgable total organic carbon after catalytic combustion at 720 °C by Shimadzu TOC-L.

For the identification of individual products of ATV photodegradation, ultra-high-performance liquid

chromatography coupled with a high-resolution mass spectrometer for detection (UHPLC-HRMS/MS) was adopted. The photoproducts mixture (P-ATV) was analysed using a Thermo Scientific Dionex UltiMate 3000 ultra-high-performance liquid chromatography plus (UHPLC+) instrument equipped with a diode array detector connected to a Bruker Impact HD (Bruker, Billerica, MA, USA) high-resolution mass spectrometer with electrospray ionisation and QTOF analyzers. Separation of photoproducts was performed on Arion Plus C18 column (3 µm; 150 mm × 4.6 mm) using (A) acetonitrile containing 0.1% formic acid and (B) water + 0.1% formic acid as a mobile phase with a 0.6 ml/min flow rate. The gradient was as follows: A/B (v/v) 15/85 (0 min), 15/85 (3 min), 100/0 (23 min), 100/0 (28 min), and 15/85 (33 min). The volume of sample injection was 5 µl. The parameters of the ion source and MS/MS experiment are given in Table 1. The obtained spectra were calibrated using the Lock Mass Tuning Mix ES-TOF internal calibration standard (622 Da) and sodium formate clusters at the beginning of each analysis using Bruker Compass Data Analysis 5.1 and Bruker Compass Library Editor 5.1 software.

Toxicity assays

The ecotoxicity of both the ATV and P-ATV was evaluated using a series of toxicity assays on three model organisms representing different levels of the aquatic food web, an alga, a crustacean, and fish embryos.

Desmodesmus toxicity assay

The growth inhibition test based on OECD methodology 201 (OECD 2011) was used to assess the toxicity of ATV and P-ATV on algae. The stock solutions of ATV and P-ATV were pre-diluted in Z medium (growth medium used for cyanobacteria and algae cultivation according to Staub 1961). The initial cell concentration of the model organism *Desmodesmus subspicatus* (obtained from CCALA Třeboň, Czech Republic, https://ccala.butbn.cas.cz/en; strain number BRINKMANN 1953/SAG 86.81) was 4×10^3 cell/ml. The growth inhibition test was done in 96-well microplates. Test concentrations were arranged in a geometric series with a factor of 4. As a control, only the Z medium with the same amount of *Desmodesmus* cells was used. All microplates were incubated in Climacell EVO line incubator tempered to a constant temperature of 23.0 ± 0.1 °C with constant irradiation. The growth of the alga was quantified as a fluorescence signal using a BioTek Cytation 5 microtitration plate reader (excitation wavelength: 485 nm, emission wavelength: 680 nm) every 24 h for 72 h. Shaking of the microplate was applied prior to the fluorescence reading.

Daphnia toxicity assays

To evaluate the possible effect of ATV and P-ATV on *Daphnia magna*, both the short-term acute and long-term chronic toxicity assays were performed according to OECD 202 (OECD 2004) and OECD 211, (OECD 2012). The stock solutions of ATV and P-ATV were diluted in ADaM (Aachener Daphnien Medium, which is based on synthetic sea salt and analytical grade chemicals added to deionized water according to Klüttgen et al. 1994).

In the acute toxicity assay, two neonates of *D. magna* not older than 24 h were introduced into 10 ml of test concentrations, with the ADaM medium serving as a control. Acute toxicity was performed in at least five replicates for each concentration of ATV or P-ATV. The samples were

Table 1 Parameters of ion source and MS/MS experiment

Ion source	
Dry temperature	250 °C
Drying gas flow	11 l/min
Nebulizer	3 bar
Capillary voltage	4200 V
Endplate offset	− 500 V
Collision gas supply	35%
Quench time	5 ms
Collision energy (MS experiment)	10 eV
MS/MS experiment	
Identical instrument settings	
Collision energy (MS experiment)	70 eV
Range of m/z	20–2200
Spectra rate loading	1.00 spec/s

kept in an incubator (temperature 20.2 ± 0.2 °C, photoperiod 16 h light and 8 h dark). The daphnids were not fed during the acute assay and their immobilisation was checked after 24 and 48 h.

Chronic exposure to ATV and P-ATV was performed according to OECD 211. Female neonates – daphnids younger than 24 h — were individually introduced into 50 ml of media in three different treatments (ATV, P-ATV, and the control), each of the treatments in 15 replicates. The test lasted 21 days, with the condition of incubation the same as in the acute test. The daphnids were fed every second day with the green alga *Desmodesmus subspicatus* (3 mg C/l), the medium was changed every four days. The survival and number of offspring were monitored every other day.

Danio embryos toxicity assay

The toxicity assay on *Danio rerio* embryos followed the OECD 236 method (2013). The stock solutions of ATV and P-ATV were diluted in ISO water (ISO 2007). Newly fertilised eggs were selected under a stereomicroscope and transferred into 2 ml of a particular concentration of ATV or P-ATV in the 24-well microplates. Every microplate represented one concentration resulting in 24 replicates. Pure ISO water was used as a control. The test was performed in an incubator tempered at 24.9 ± 0.2 °C with a photoperiod of 13 h of light and 11 h of dark for 96 h, with one exchange of the incubation media after 48 h. Coagulation of fertilised eggs, lack of somite formation, lack of detachment of the tail from the yolk sac, and a slow heartbeat, if present, were recorded as indicators of lethality every 24 h; morphological and behavioural abnormalities were checked using a stereomicroscope.

Statistical analysis

The growth inhibition of algae (immobilisation of *Daphnia*, and mortality rate of *Danio* embryos) was calculated as a measure of the effect of both ATV and P-ATV. The values of IC20 (Inhibitory Concentration that causes inhibition of 20% of organisms) and LC50 (Lethal Concentration required to kill 50% of test organisms) of all three tested organisms were obtained; since the inhibition curve has a sigmoidal profile, the non-linear regression fitting using the GraphPad Prism 7.05 software package (GraphPad Software, www.graphpad.com) was adopted for the procedure. Differences in the number of juveniles and clutches, interclutch period, and body size in the long-term toxicity assay on *D. magna* were assessed by the Kruskal–Wallis test followed by *post-hoc* comparisons using Statistica 14 (TIBCO Software Inc. 2020). In zebrafish embryos, mortality at 96 hpf, hatching rate and abnormalities for the whole experimental period were assessed by the generalised linear model (GLM) with an assumed binomial distribution using R (R Core Team 2023). GLM models including substance (ATV vs. P-ATV), concentration levels and interactions between substance and concentrations were built. The best-fitting model (including substance, concentration and interaction) based on the Akaike information criterion (AIC, a technique based on in-sample fit to estimate the likelihood of a model to predict/estimate the future values) is presented. The treatment effect on zebrafish mortality, hatching rate, and abnormalities was tested by a chi-square test.

Results and discussion

Kinetics of ATV photodegradation

ATV itself is not degraded readily. Since surface waters usually contain metal ions, especially ferric ions, that may act as a photocatalyst, Fe (III) ions presence was tested and shown beneficial. Photochemical degradation of ATV in the presence of the photocatalyst Fe (III) in the concentration of 5 mg/l followed first-order kinetics with the rate constant k equal to 0.130 min^{-1}. After 15 min of irradiation, 85% of the original amount of ATV was degraded, and all main products detectable on the HPLC chromatogram with fluorescence detection were well developed.

TOC analysis revealed that irradiation up to 25 min (when all of the ATV present in the reaction mixture was degraded) did not result in any decrease in organic carbon in the reaction mixture, thus proving that no mineralisation (degradation of organic carbon to CO_2) occurred under the irradiation conditions used in the study.

Identification of P-ATV

Fourteen major photoproducts were detected in the irradiated ATV solution by UHPLC-HRMS/MS analysis. Their m/z values, intensity in the mass spectrum, retention times, calculated ion formula with errors in ppm, and proposed structure are listed in Table 2.

All formed photoproducts had higher retention times than the parent compound which indicated that they were less polar than ATV itself. The two most intensive photoproducts were product 6 (m/z 515.2704; rt 19.0 min; intensity 2.9×10^5) and product 2 (m/z 511.2386; rt 24.8 min; intensity 1.00×10^5), which were formed by decarboxylation and phenanthrene structure formation. Other photodegradation pathways included dehydration, oxidation, ether group formation, and dehydrogenation to form a highly conjugated system. For products 2; 3; 4; 5; 6; 8; 9; 10; 11; 13 and 14 pathways of photodegradation mechanism were proposed (Fig. 1).

Table 2 Atorvastatin and its major photoproducts (sorted in ascending order by m/z) detected in the ATV solution after 15 min of irradiation. Abbreviations: *rt* retention time, *err* error.

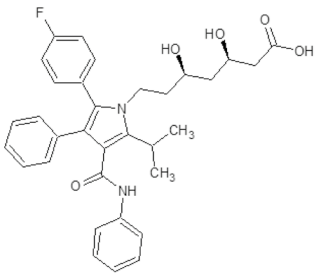
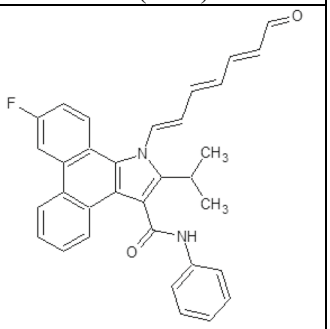
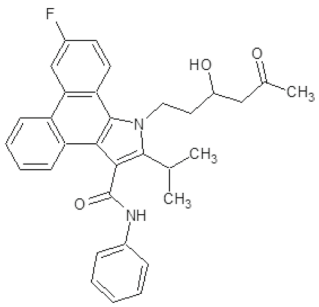
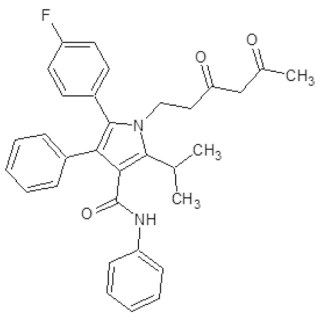
m/z	intensity in MS	rt [min]	ion formula	err [ppm]	structure
559.2603	2×10^5	17.4	$C_{33}H_{36}FN_2O_5$	-0.3	 atorvastatin (ATV)
503.2106	2.8×10^4	20.8	$C_{33}H_{28}FN_2O_2$	4.6	 product 1
511.2386	1.0×10^5	24.8	$C_{32}H_{32}FN_2O_3$	2.3	 product 2
511.2396	3.2×10^4	21.7	$C_{32}H_{32}FN_2O_3$	-2.3	 product 3

Table 2 (continued)

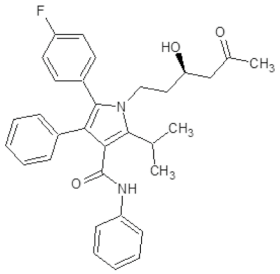
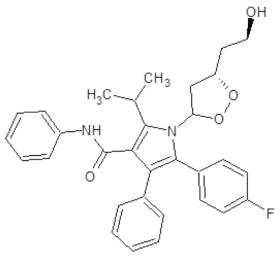
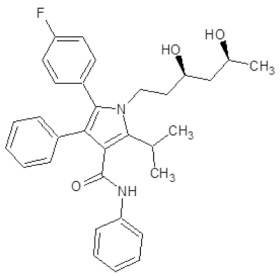
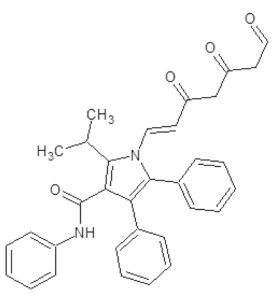
m/z	intensity in MS	rt [min]	ion formula	err [ppm]	structure
513.2557	4.1×10^4	19.5	$C_{32}H_{34}FN_2O_3$	2.6	 product 4
515.2344	2.05×10^4	18.1	$C_{31}H_{32}FN_2O_4$	-3.7	 product 5
515.2704	2.9×10^5	19.0	$C_{32}H_{36}FN_2O_3$	-0.7	 product 6
519.2043	0.5×10^4	20.7	$C_{33}H_{28}FN_2O_3$	5.1	not identified product 7
519.2425	0.65×10^5	21.2	$C_{33}H_{31}N_2O_4$	-27.8	 product 8

Table 2 (continued)

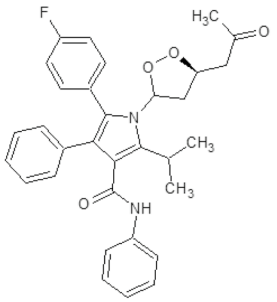
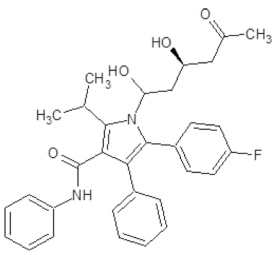
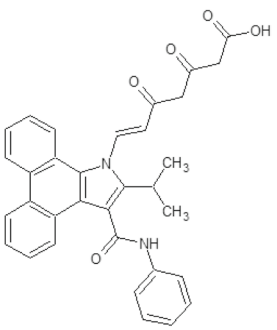
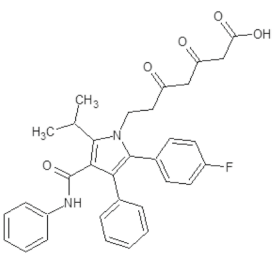
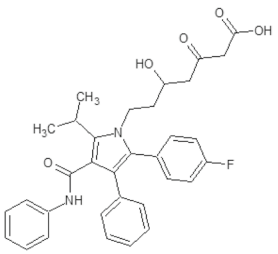
m/z	intensity in MS	rt [min]	ion formula	err [ppm]	structure
527.2441	0.3×10^4	20.1	$C_{32}H_{32}FN_2O_4$	9.9	 product 9
529.2510	1.15×10^4	17.7	$C_{32}H_{34}FN_2O_4$	12.2	 product 10
533.2211	1.75×10^4	22.4	$C_{33}H_{29}N_2O_5$	-5.6	 product 11
545.2433	1.0×10^4	17.9	$C_{32}H_{34}FN_2O_5$	-6.9	not identified product 12
555.2285	8.5×10^3	18.5	$C_{33}H_{32}FN_2O_5$	9.1	 product 13

Table 2 (continued)

m/z	intensity in MS	rt [min]	ion formula	err [ppm]	structure
557.2442	1.7×10^4	18.2	$C_{33}H_{34}FN_2O_5$	6.1	 product 14

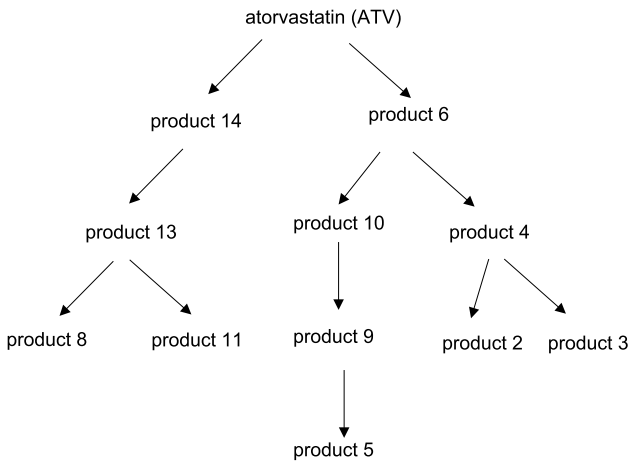


Fig. 1 Proposed degradation pathways of atorvastatin (ATV) under irradiation

Results of toxicity tests

Since ATV was degraded in the presence of ferric ions, the effect of the ferric ions on the tested organisms was examined. The results confirmed that ferric ions did not affect the tested organisms in concentrations applied in this study.

For the P-ATV toxicity tests, the solution of ATV irradiated for 15 min was used, because there was a remaining amount of ATV (15% of the original amount), and the products detectable by fluorescent detector were well developed. Thus, after 15 min of irradiation, the amount of ATV decreased 6.7 times. To ensure that in the tests comparing P-ATV and ATV the concentration of ATV was the same, a 6.7 times larger volume of the irradiated mixture of P-ATV was always added to the incubation medium.

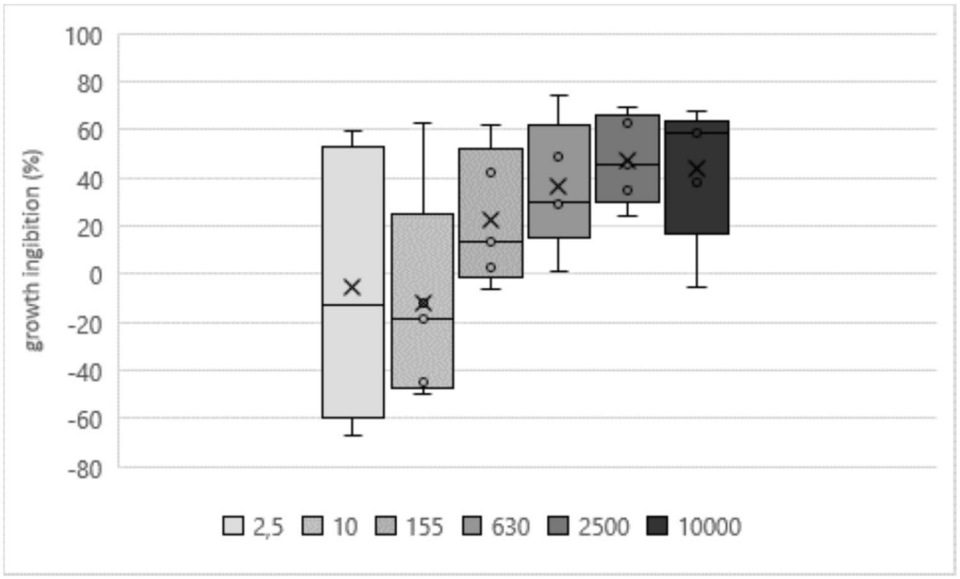
The effect of ATV and P-ATV on *Desmodesmus*

Figure 2 shows that both ATV and a mixture of its photoproducts affect negatively the growth of *D. subspicatus*. With ATV itself, the IC20 value was estimated to be 140 µg/l under the assumption of linearity of inhibition between 10 and 155 µg/l; for P-ATV, the determined IC20 value was 475 µg/l under the assumption of linear inhibition between 300 µg/l and 1200 g/l.

The effect of ATV and P-ATV on *Daphnia*

ATV itself showed a lower toxicity effect (LC50 = 38.5 mg/l) compared to a mixture of photoproducts. (LC50 = 3.25 mg/l) on the cladoceran model *D. magna* (Fig. 3). The long-term exposure was done in two concentrations of ATV and P-ATV. In the concentration of 200 µg/l, high *Daphnia* mortality was observed in photoproducts: no individual produced a clutch, they died on the 9th day of the experiment before they were able to produce any juveniles. No mortality was observed in the control and ATV treatment. Data presented in Fig. 4 were obtained in the experiment where the ATV concentration used was set to 50 µg/l. In this concentration, chronic exposure revealed a negative effect of P-ATV on the number of juveniles (Kruskal–Wallis test, $H = 22.3$, d.f. = 2, $P < 0.001$) and body size (Kruskal–Wallis test, $H = 23.8$, d.f. = 2, $P < 0.001$) of *Daphnia*—both of these parameters were significantly lowered (Fig. 4A, D). ATV did not affect any parameter and obtained results were comparable with the control (Fig. 4A, D). Interclutch period and number of clutches were not affected regardless of whether *Daphnia* was exposed to the parental substance or.

P-ATV (Fig. 4B, C).



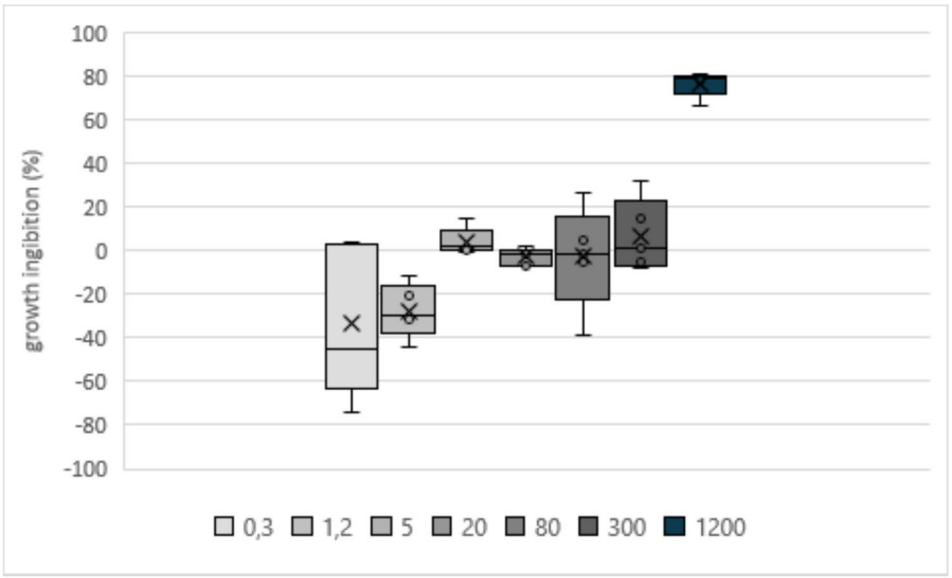
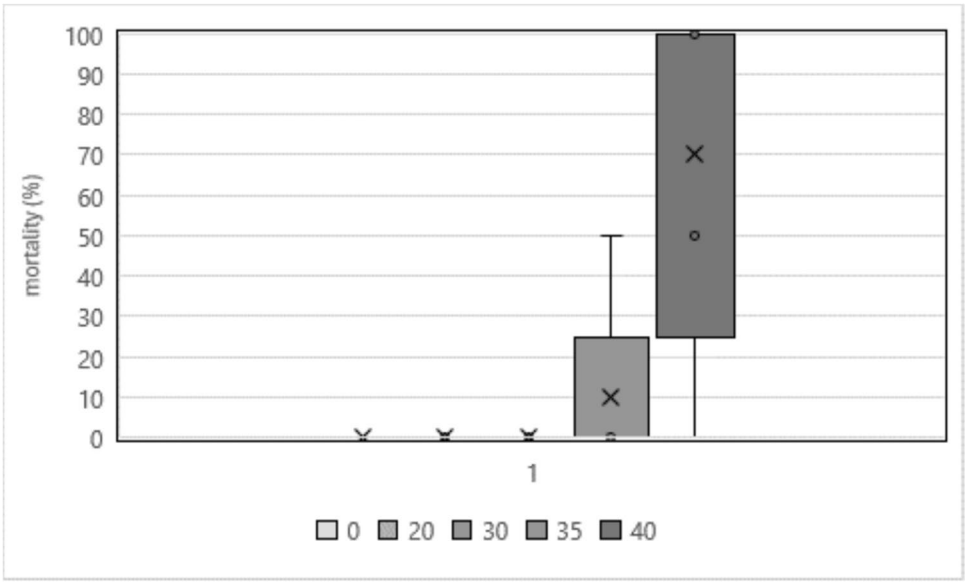


Fig. 2 Growth inhibition in *Desmodesmus*: upper part – ATV, lower part—P-ATV. The numbers in legends give the concentrations in µg/l

The effect of ATV and P-ATV on *Danio*

The LC50 after 96 h post fertilisation (hpf) exposure to ATV (5.56 mg/l) was twice higher compared to P-ATV (2.74 mg/l) as demonstrated in Fig. 5. Only one zebrafish embryo died in the control treatment at 24 hpf while no embryo survived until 96 hpf in the highest tested concentration of both ATV and P-ATV (Fig. 6). Most hatched embryos occurred at 72 hpf (Fig. 5). Mortality was significantly dependent on the substance ($\chi^2 = 4.133$, $P = 0.04$), concentration ($\chi^2 = 279.126$, $P < 0.001$, with the growing concentration the increasing the probability of mortality) and the interaction between substance and concentration ($\chi^2 = 26.821$, $P < 0.001$). The hatching rate increased with increasing ATV concentration except for the highest concentration, which was marked by most dead hatched embryos (Fig. 5). Although the effect of the substance itself was not significant, hatching significantly depended on concentrations ($\chi^2 = 108.223$, $P < 0.001$) and the interaction between the substance and concentration ($\chi^2 = 27.652$, $P < 0.001$). Embryos exposed to ATV revealed a higher proportion of malformations at a concentration equal to 2.5 mg/l or higher. In P-ATV, abnormalities were generally observed in even lower concentrations compared to ATV. The most common malformation was represented by pericardial oedema (e.g. 50% in 2.5 mg/l of ATV; 79% in 10 mg/l of ATV). A few embryos with pericardial oedema (13%) were observed in the concentration of 1 mg/l but up to 70% in higher concentrations of P-ATV. Generally,



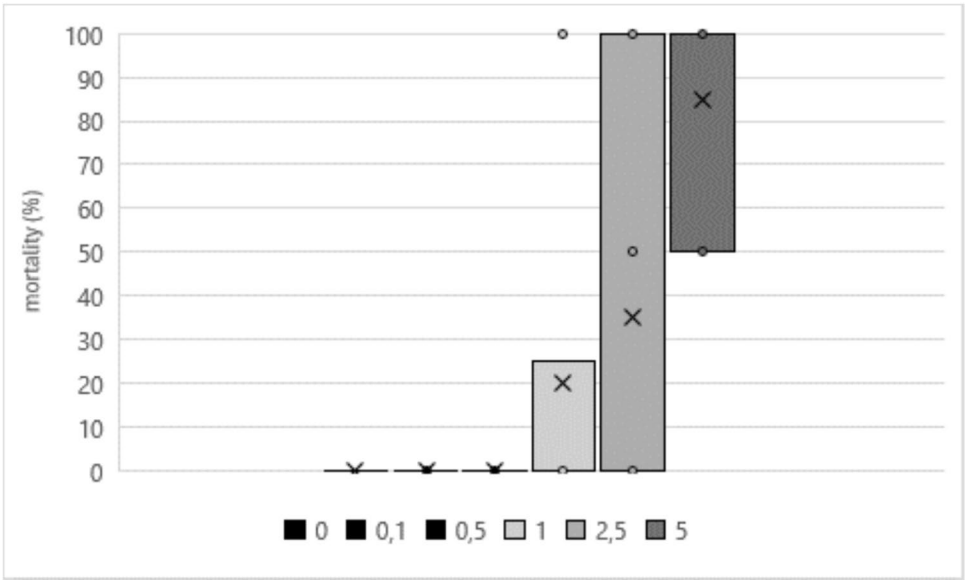


Fig. 3 The effect of ATV (upper part) and P-ATV (lower part) on *D. magna* after 48-h exposure. The numbers in legends give the concentration in mg/l

the probability of pericardial oedema was related to the substance ($\chi^2 = 8.920$, $P = 0.01$), concentration ($\chi^2 = 213.908$, $P < 0.001$), and their interaction ($\chi^2 = 33.566$, $P < 0.001$). The presence of pericardial oedema in both the ATV and P-ATV differed from the control in all concentrations higher than 2.5 mg/l (Fig. 6). Exposure to 10 and 25 mg/l of ATV resulted in a significant increase of spine malformation which was not found in P-ATV. Therefore, the effect of substance ($\chi^2 = 16.082$, $P < 0.001$), concentration ($\chi^2 = 80.813$, $P < 0.001$) but not the interaction ($\chi^2 = 1.770$, $P = 0.97$) on the probability of spine malformation was found. Moreover, an exposure to 10 mg/l increased yolk sac oedema incidence.

Discussion

The present study showed that ATV undergoes photochemical degradation under light conditions relevant to surface waters; when ferric ions, the ions usually occurring in freshwaters (Dojlido and Best 1993), are added to the reaction mixture in concentration commonly found in freshwater (mg/l), the reaction is facilitated.

The UHPLC-HRMS/MS analysis revealed fourteen photoproducts with high-intensity signals. Their m/z values, elements composition, and supposed resulting structures formed by decarboxylation, phenanthrene structure

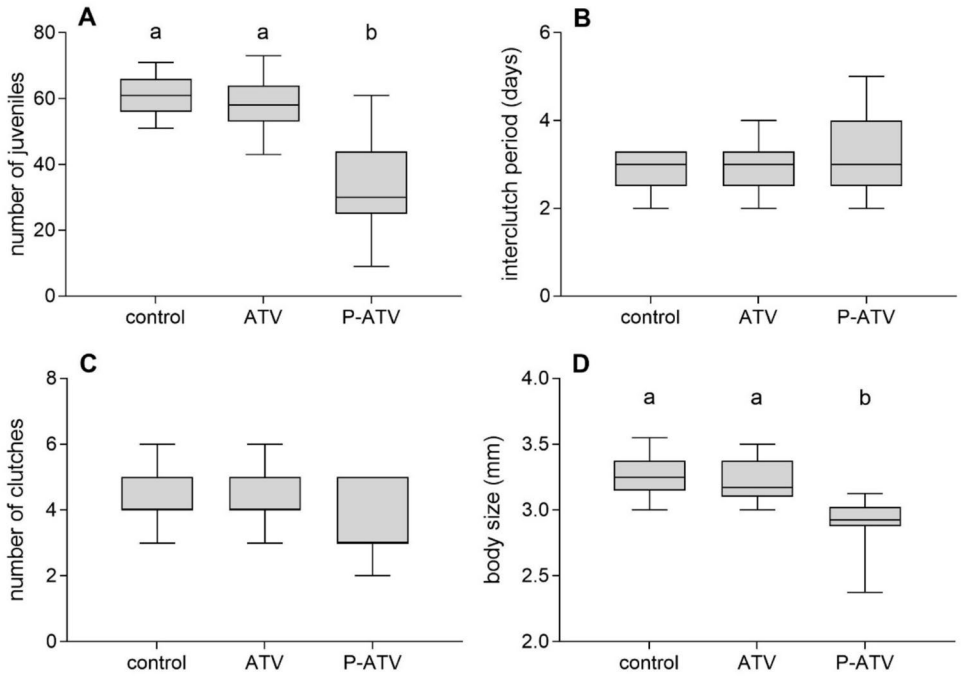


Fig. 4 The results of the long-term chronic toxicity assay using *Daphnia magna*. Number of juveniles (**A**), interclutch period (**B**), number of clutches (**C**), and body size (**D**) after 21 days of the experiment. Boxes show the 25th and 75th percentiles with median; whiskers represent the range (min–max). ATV – atorvastatin (50 µg/l); P-ATV – mixture of photoproducts (the remaining concentration of ATV 50 µg/l). Significant differences are indicated by indices

formation, dehydration, dehydrogenation, and ether group formation are in agreement with the studies of Montanaro et al. (2009) and Ping et al. (2021).

The toxicity assays were performed on aquatic organisms of three trophic levels, since a combination of assays on organisms of different trophic levels may provide a more complex picture of the potential negative effects of studied substances. In this study, the influence of ATV and P-ATV on three different aquatic organisms, an alga, a crustacean, and a fish embryos, was investigated.

The toxicity assays disclosed different effects on individual tested organisms:

The growth of *D. subspicatus* was inhibited by ATV more than by the photodegradation reaction mixture (Fig. 2). Since the concentration of ATV was the same in the ATV and P-ATV samples, it may be concluded that conjugation fo ATV with some of the P-ATV components is responsible for the lowering of toxicity in the P-ATV sample.

A different trend was observed in the trial with *D. magna* and with embryos of *D. rerio.*

In the acute toxicity test with *D. magna*, the P-ATV was almost 12 times more toxic than ATV (LC50 values for P-ATV and ATV being 3.25 mg/l and 38.5 mg/, resp., as shown in Fig. 3).

Since environmentally relevant concentrations of ATV are up to the low hundreds of µg/l (Kleywegt et al. 2019; Tete et al. 2020; Goswami et al. 2022), the value of 200 µg/l was chosen for the chronic toxicity study on *D. magna*. In this test, no effect was observed for ATV itself while the exposure to a P-ATV mixture with the same remaining ATV concentration resulted in the dying of all daphnids in 9 days of exposure before they were able to produce any juveniles. In P-ATV with the ATV remaining concentration of 50 µg/l, a significantly lowered number of juveniles and an increased number of males in neonates were observed in the 21 day chronic test (Fig. 4).

With *D. rerio*, the LC50 determined value for ATV was 5.6 mg/l, which is in agreement with the results obtained by Kingcade et al. 2021). P-ATV exhibited twice higher toxicity with LC50 value of 2.7 mg/l (Fig. 5). Higher incidence in sublethal malformations was observed in P-ATV than ATV after exposure to the medium with 2.5 mg/l of ATV or remaining ATV in P-ATV:

heart oedema (48% of embryos exposed to 2.5 mg/l of ATV, 77% for P-ATV), yolk sac eodema (10% and 35% for ATV and P-ATV, resp.), and spine malformation such as bent spine (0% and 8% for ATV and P-ATV, resp.). Similar malformations were observed by Ping et al. (2022) after exposure of *D. rerio* embryos to ATV. Nevertheless, environmentally relevant concentrations did not result in a statistically significant occurrence of malformations in either ATV or P-ATV. (Fig. 5).

Regarding the possible mechanism by which ATV affects (and P-ATV might affect) aquatic organisms, it has been conclusively demonstrated that ATV as well as other statins alters and disrupts lipid metabolism in aquatic invertebrates (Falfushynska et al. 2019) as well as in aquatic vertebrates (Santos et al. 2016; Wang et al. 2021; Yufei Zhao et al. 2024b).

Since individual P-ATV identified in this study have longer retention time and are therefore even less polar than ATV, it can be assumed that they also can interfere with lipid

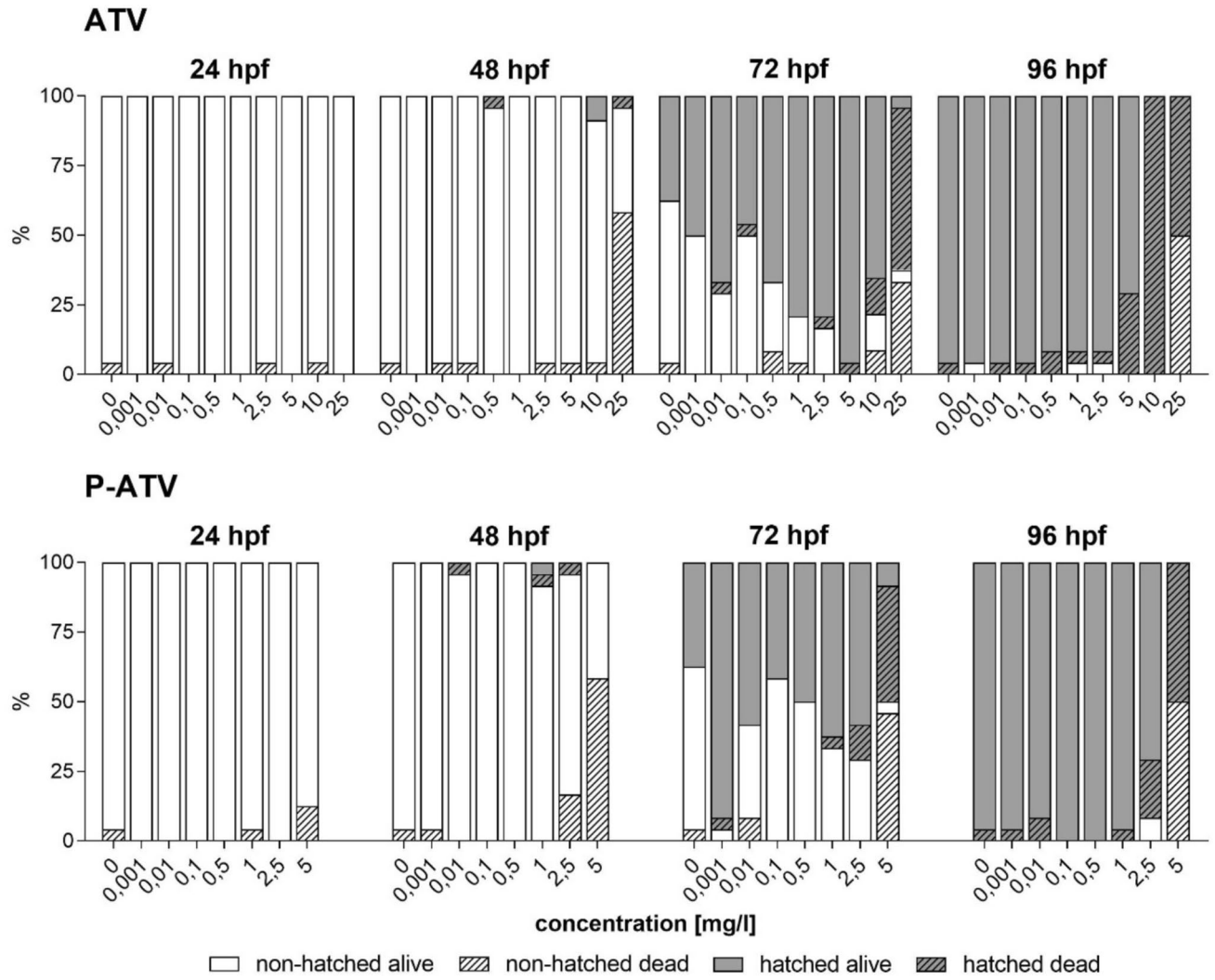


Fig. 5 *Danio rerio* mortality and hatching after 24, 48, 72 and 96 hpf for both the atorvastatin (ATV) and photoproducts (P-ATV)

metabolism even though not via the inhibition of a specific enzyme of cholesterol synthesis as atorvastatin and other statins do in all vertebrate animals (Santos et al. 2016).

Thus, ATV and its photoproducts may pose a threat to aquatic organisms of all trophic levels because lipids are fundamental components of all living creatures. If an accumulation of these compounds in the lipidic tissues of fish occurs, it may cause a hazard even for humans.

A systematic analysis of 322 studies about the environmental occurrence and effects on aquatic organisms provided by Zhang et al. (2020) concluded that knowledge of the environmental implications of lipid-regulating drugs is limited and multiple endpoints of influence on physiological alteration with health consequences may be expected.

An investigation of photochemical transformations of pharmaceuticals under light and other conditions relevant to freshwater bodies was a discipline with limited awareness up to two decades ago (Tixier et al. 2002; Tixier et al. 2009) that started attracting attention only recently (Traviński and Skibiński 2017; Klementová et al. 2020; Klementová et al. 2021; Klementová et al. 2022; Traviński and Skibiński 2022).

The presented paper tried to contribute to the understanding of the fate and implications of pharmaceuticals on the organisms in the aquatic environment by considering and investigating not only one of the top ten parental drugs, atorvastatin, itself but also its possible photochemical transformation under environmentally relevant conditions and the influence of the photoproduct mixture on selected aquatic organisms.

Conclusion

Photodegradation of ATV under the light conditions relevant to natural surface waters and in the presence of the commonly occurring metal (ferric ions) led to 14 major

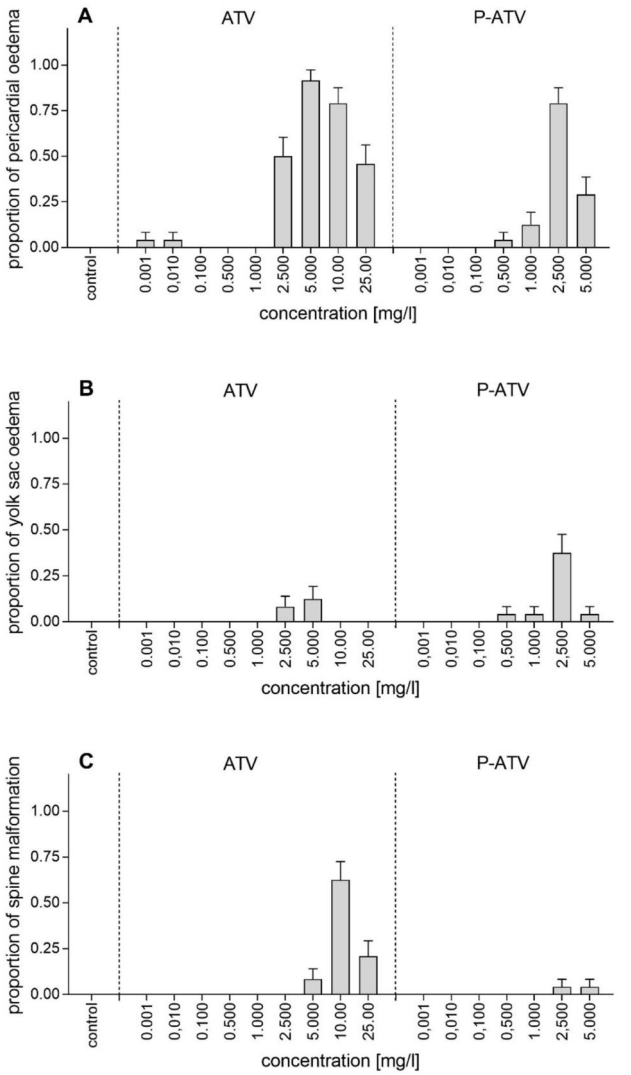


Fig. 6 Malformations observed after *Danio rerio* exposure to atorvastatin (ATV) and a mixture of its photoproducts (P-ATV) during the whole assay. Data represent mean ± SEM of the proportion of oedemas and spine malformation (the number of affected embryos/all embryos)

photoproducts formed by decarboxylation, dehydration, dehydrogenation, oxidation, and phenanthrene-like structure formation. For 12 of the major photoproducts, structures were suggested.

Both ATV and a P-ATV mixture had negative effects on the tested organisms: regarding *D. subspicatus*, ATV inhibited the growth of the alga much more than the P-ATV mixture at the same ATV concentration, which may be the result of conjugation of photoproducts to ATV.

The opposite effect was seen in the assays with *D. magna* and *D. rerio*; in both these cases, P-ATV was demonstrated to be more detrimental than ATV itself (ca twelve times for daphnia and two times for fish embryos).

With *D. magna*, ATV did not exhibit any effect up to the concentrations of 200 µg/l; however, P-ATV with the remaining concentration of atorvastatin of 200 µg/l led to the death of all daphnids before they were able to produce juveniles. Long-term exposure to P-ATV with the concentration of ATV of 50 µg/l significantly (ca twice) lowered the number of juveniles, the number of juveniles per brood, and the size of the adult females.

With *D. rerio* embryos, the environmentally relevant concentrations exhibit no effect. In higher concentrations, the presence of both ATV and P-ATV resulted in malformations such as heart oedema, yolk sac oedema, and bent spine, the occurrence of malformations being higher in P-ATV in comparison with ATV (heart oedema 1.4 times and yolk sac oedema 3.5 times after 96 h exposure in 2.5 mg/l, bent spine observed only in P-ATV in 8% of embryos). After 72 h exposure to the concentration of 2.5 mg/l, ca 5% of embryos were dead in ATV while 19% in P-ATV; after 96 h exposure in the same concentration, 7% of embryos were dead in ATV and almost 30% in P-ATV.

The novel and original result of the study is a clear indication that the P-ATV mixture has more pronounced deleterious effects than the parent compound ATV, with daphnids even in environmentally relevant concentrations. This may affect higher trophic-level organisms that rely on daphnids as food. The future investigation of the individual compounds in the mixture and their separate effects on daphnids is advisable for a better understanding of possible outcomes of atorvastatin-contaminated surface waters.

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Author contributions All authors contributed to the study conception and design. Martina Poncarová, Michal Šorf, Eva Poštulková and Denisa Medková are responsible for the toxicity tests on tested organisms. Šárka Klementová, Pavla Petráňová and Pavla Fojtíková analysed the irradiated mixture. David Kahoun, Jan Hájek and Pavel Hrouzek validated the method on HPLC-HRMS/MS. The first draft of the manuscript was written by Martina Poncarová and all authors commented on previous versions of the manuscript. All authors have read and approved the final manuscript.

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Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

Ethical approval Not applicable.

Consent to participate Not applicable.

Consent to publish The authors declare that they have no competing interests.

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