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ORIGINAL ARTICLE

Rapid ecotoxicity and genotoxicity assessment using *Macropodus ocellatus* cells

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Abstract

Toxic substances can cause serious harm to aquatic organisms and humans who consume them. Rapid ecotoxicity assessment and genotoxicity assessment should be performed simultaneously to detect potential harm caused by toxic substances. In a previous study, an ecotoxicity and genotoxicity assessment system was established by treating fish cells derived from *Cyprinus carpio* (*C. carpio*) with toxic substances in a medium containing 1% fetal bovine serum (FBS) for 6 h. In this study, these conditions (1% FBS/6 h) were applied to fish cells derived from *Macropodus ocellatus* (*M. ocellatus*). Surprisingly, the new assessment tool using *M. ocellatus* cells provided ecotoxicity and genotoxicity data similar to those of *C. carpio* cells. In addition, the new assessment tool demonstrated its suitability as an assessment platform by demonstrating ecotoxicity and genotoxicity for substances known to be genotoxic (fluxapyroxad, fipronil, clarithromycin, 2,4-di-tert-butylphenol, perfluorooctanoic acid, prochloraz, abamectin, and climbazole). In conclusion, this study established an ecotoxicity and genotoxicity assessment system that can rapidly generate data. This assessment platform can be used as a tool to analyze a large number of toxins within a given period of time.

Keywords Ecotoxicity assessment · Genotoxicity assessment · *Macropodus ocellatus* · Neutral comet assay

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Introduction

Toxicants from environmental and industrial pollutants can affect aquatic life [1]. Accumulation of toxicants in aquatic organisms can have negative effects on larvae and embryos. It can cause morphological changes, spinal deformation, increased mortality, and decreased cardiac activity [1]. People who eat aquatic organisms can also be affected, causing waterborne diseases, immune system suppression, and reproductive dysfunction [2]. Among the toxicants, genotoxins can accumulate in aquatic organisms and damage DNA, causing morphological or functional defects [3]. An effective monitoring platform to assess the ecotoxicity or genotoxicity of toxicants is essential to protect aquatic organisms.

Cells derived from fish are being used as an alternative to fish-based ecotoxicity assessments by eliminating variations due to various responses of fish [4]. The semi–effective concentration (EC_{50}) using the rainbow trout-derived RTG-2 cells significantly correlated with the lethal concentration 50 obtained using rainbow trout, indicating that ecotoxicity assessment using RTG-2 cells can be applied as an alternative platform to estimate the ecotoxicity of toxicants to fish

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[5, 6]. However, the assessment period using fish cells varies from 4 to 21 days depending on the experimental goals [7, 8], which restricts the quantity of toxicants that can be evaluated in a specific amount of time.

Fish-derived cells have been also employed as surrogates for fish in genotoxicity experiments because they are sensitive to low doses of genotoxins [9]. For example, the genotoxicity of ethyl methanesulfonate and benzo[a]pyrene (B[a]P) was first demonstrated using the PLHC-1 cells derived from *Poeciliopsis lucida* [10]. In addition, RTG-2 cells have been used as a useful tool for monitoring genotoxicity through the assessment of various toxicants [11–13]. However, fish-derived cells require toxicity treatment for 1–36 days for genotoxicity assessment, leaving room for improvement [4].

Recently, a platform capable of assessing both ecotoxicity and genotoxicity within 6 h using *C. carpio* cells has been developed [14, 15]. This platform is suitable for situations requiring rapid assessment and presents a new paradigm for ecotoxicity and genotoxicity assessment [14, 15]. If a platform capable of rapidly measuring genotoxicity in fish cells other than *C. carpio* cells is developed, its use in conjunction with *C. carpio* cells could provide more accurate ecotoxicity and genotoxicity data.

Macropodus ocellatus (*M. ocellatus*) is found in East Asia, including China, Japan, and Korea [16]. An ecologically important species, *M. ocellatus* can be found in a variety of habitat types and ecological environments. Additionally, because it responds rapidly to water pollution, *M. ocellatus* functions as an important indicator for evaluating environmental toxicity [16].

In this study, we established a rapid ecotoxicity and genotoxicity assessment platform using *M. ocellatus* cells. Two parameters that allow rapid ecotoxicity and genotoxicity assessment using *C. carpio* cells were applied to *M. ocellatus* cells, allowing similar assessments to *C. carpio* cells. Herein, we present a novel platform utilizing *M. ocellatus* cells that could become a key tool to meet the increasing demand for ecotoxicity and genotoxicity.

Materials and method

M. ocellatus cells

M. ocellatus cells were isolated from *M. ocellatus* fish according to the method used in previous studies [14, 15]. *M. ocellatus* cells showed fibroblast morphology, which was consistent with previous studies [14, 15]. Dulbecco's Modified Eagle's medium supplemented with 25 mM glucose, 20% fetal bovine serum (FBS; SH30919.03; Hyclone, Waltham, MA, USA), 0.2 mg/ml primocin (ant-pm-1; Invivogen, San Diego, CA, USA), 1% L-glutamine (SH3003401; Hyclone), and 0.5% HEPES (15,630–106; Gibco, Waltham, MA, USA) was used. *M. ocellatus* cells were cultured at 25 °C, in an atmosphere of 5% CO_2 and 20% O_2. The Animal Experiment Ethics Committee at Incheon National University approved all studies with *M. ocellatus* fish and *M. ocellatus* cells (Protocol number: 2025-01-2862).

Ecotoxicity assessment

Ecotoxicity evaluation was performed by seeding 2×10^3 cells per well in 96–well plates and culturing them for 6 h in medium containing 1% FBS, following the protocol proposed in our previous study [15]. Cells were then exposed to toxicants at concentrations of 62.5, 125, 250, 500, and 1000 ppm. The tested substances included butachlor (37,887; Sigma, St. Lous, MO, USA), 2,4,6–trichlorophenol (3543; Sigma), roxithromycin (R1500000; Sigma), fluxapyroxad (37,047; Sigma), fipronil (46,451; Sigma), clarithromycin (C9742; Sigma), 2,4–Di–tert–butylphenol (137,731; Sigma), perfluorooctanoic acid (171,468; Sigma), prochloraz (45,631; Sigma), abamectin (31,732; Sigma), and climbazole (1,135,600; Sigma). For each condition, the experiment was repeated three times, resulting in three independent biological data points (biological triplicates). Each of biological triplicates was derived from analyzing 3 different samples. Furthermore, biological triplicates were reported using mean and 95% confidence interval (CI).

Genotoxicity assessment

6-well plates containing media supplemented with 1% FBS were used to seed *M. ocellatus* cells. Each well had a cell density of 7×10^4. Then, cells were exposed to toxicants for 6 h. R&D Systems, Minneapolis, MN, USA, provided the CometAssay Single Cell Gel Electrophoresis Assay Kit (4250–050–K). ImageJ (National Institutes of Health, Bethesda, MD, USA) software was used to quantify the lengths of individual DNA comets in pixels. For the dimethyl sulfoxide (DMSO) control, DMSO (D8418; Sigma) was added to the medium at a final concentration of 0.01%. For each condition, the experiment was repeated three times, resulting in three independent biological data points (biological replicates). Each of biological triplicates was derived from analyzing 23 different samples. Furthermore, each of biological triplicates was reported using mean and standard deviation.

Statistical analyses

Two-way ANOVA followed by Bonferroni's post hoc test were performed using a GraphPad Prism 9 (San Diego, CA, USA). The no observed effect concentration (NOEC), 10%

effect concentration (EC_{10}), a semi–effective concentration (EC_{50}), and 95% confidence interval (CI) were calculated using a GraphPad Prism 9.

Results

Comparison of ecotoxicity and genotoxicity of butachlor using *C. carpio* and *M. ocellatus* cells

Butachlor (CAS number: 23184–66–9; $C_{17}H_{26}ClNO_2$; molecular weight: 311.8 g/mol) is a phenylacetamide–type herbicide primarily used for weed control in rice fields (Fig. 1A) [17]. It contaminate aquatic organisms and threaten aquatic ecosystems and environments that ingest them [18].

In the previous study, we performed an ecotoxicological evaluation using *C. carpio* cells by applying two parameters: toxicant treatment for 6 h in medium containing 1% FBS [15]. This ecotoxicological evaluation generated data more quickly than the traditional evaluation [15]. Therefore, we investigated whether the ecotoxicological evaluation applying these two parameters to *M. ocellatus* cells would provide similar results to the ecotoxicological evaluation using *C. carpio* cells. As a control, *C. carpio* cells were cultured in media containing 1% FBS for 6 h with different concentrations of butachlor (0, 62.5, 125, 250, 500, and 1000 ppm). The effect of butachlor on cell viability displayed a sigmoid pattern and the EC_{50} value was 261.423 ± 38.125 (mean ± 95% CI) ppm (Fig. 1B). The no observed effect concentration (NOEC) of butachlor was less than 78.57 ± 24.131 (mean ± 95% CI) ppm, and the 10% effect concentration (EC_{10}) was 122.492 ± 10.493 (mean ± 95% CI) ppm (Fig. 1B). As an experimental group, *M. ocellatus* cells were administered with different concentrations of butachlor (0, 62.5, 125, 250, 500, and 1000 ppm) for 6 h in a medium containing 1% FBS. The effect of butachlor on cell viability showed a sigmoid pattern, and the EC_{50} value was 248.672 ± 27.004 (mean ± 95% CI) ppm (Fig. 1C and Table 1). The NOEC of butachlor was less than 100.996 ± 19.729 (mean ± 95% CI) ppm, and the EC_{10} was 118.63 ± 5.044 (mean ± 95% CI) ppm (Fig. 1C and Table 1). The NOEC, EC_{10}, and EC_{50} values of *M. ocellatus* cells were very similar to those of *C. carpio* cells, suggesting that the results of the ecotoxicological evaluation using *M. ocellatus* cells were similar to those of the ecotoxicological evaluation using *C. carpio* cells (Fig. 1D).

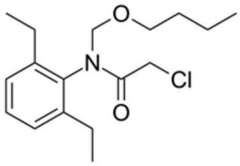
In the previous study, we also established a new genotoxicity assessment using *C. carpio* cells under the 1% FBS/6 h condition [14]. The new genotoxicity assessment also provided data more quickly compared to the traditional method [14]. Therefore, we determined to apply the 1% FBS/6 h condition to the genotoxicity test using *M. ocellatus* cells. Genotoxicity assessment uses half the EC_{50} concentration, the EC_{50} concentration, or twice the EC_{50} concentration depending on the purpose [19, 20]. Here, we performed genotoxicity assessments using half the EC_{50} concentration. As a control, *C. carpio* cells were administered with butachlor at 130.712 ppm, which is half the dose of the EC_{50} in *C. carpio* cells. In the genotoxicity assessment using *C. carpio* cells, the comet tail length of butachlor-treated cells increased by 60.7% compared to the DMSO control (Fig. 1E). As an experimental group, *M. ocellatus* cells were treated with butachlor at 124.336 ppm, which is half of the EC_{50} in *M. ocellatus* cells. In the genotoxicity evaluation using *M. ocellatus* cells, the comet tail length of the butachlor-treated cells increased by 28.5% compared to the DMSO control (Fig. 1F and Table 1). These results suggest that the genotoxicity evaluation using *M. ocellatus* cells increases the comet tail length in response to genotoxicity, similar to the genotoxicity evaluation using *C. carpio* cells.

Comparison of ecotoxicity and genotoxicity of 2,4,6–trichlorophenol using *C. carpio* and *M. ocellatus* cells

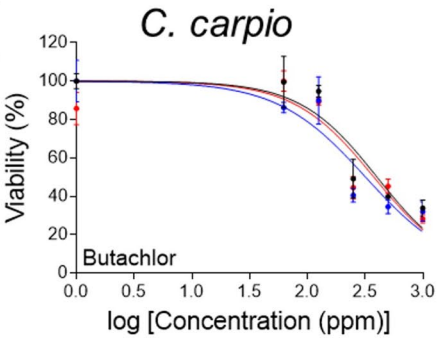
2,4,6-Trichlorophenol (CAS number: 88-06-2; $C_6H_2Cl_3OH$; molecular weight: 197.4 g/mol) has been used as an insecticide, preservative, and fungicide (Fig. 2A). This substance is found in many rivers and sediments worldwide and is resistant to degradation by aquatic organisms [21].

We verified whether the ecotoxicological evaluation using *M. ocellatus* cells showed similar results to the evaluation using *C. carpio* cells. As a control, *C. carpio* cells were treated with various concentrations of 2,4,6-trichlorophenol (0, 62.5, 125, 250, 500, and 1000 ppm) for 6 h in the medium containing 1% FBS. The effect of 2,4,6-trichlorophenol on cell viability showed a sigmoid pattern, and the EC_{50} value was 821.884 ± 28.128 (mean ± 95% CI) ppm (Fig. 2B). The NOEC of 2,4,6-trichlorophenol was less than 49.77 ± 9.976 (mean ± 95% CI) ppm, and the EC_{10} was 145.897 ± 24.524 (mean ± 95% CI) ppm, respectively (Fig. 2B). As an experimental group, *M. ocellatus* cells were treated with various concentrations of 2,4,6-trichlorophenol (0, 62.5, 125, 250, 500, and 1000 ppm) in a medium containing 1% FBS for 6 h. The effect of 2,4,6-trichlorophenol on cell viability showed a sigmoid pattern, and the EC_{50} value was 775.231 ± 7.622 (mean ± 95% CI) ppm (Fig. 2C and Table 1). The NOEC of 2,4,6-trichlorophenol was less than 102.826 ± 13.135 (mean ± 95% CI) ppm, and the EC_{10} was 165.423 ± 21.864 (mean ± 95% CI) ppm (Fig. 2C and Table 1). The NOEC, EC_{10}, and EC_{50} values of 2,4,6-trichlorophenol in *M. ocellatus* cells were very similar to those in *C. carpio* cells, suggesting that the results of the ecotoxicological evaluation

A

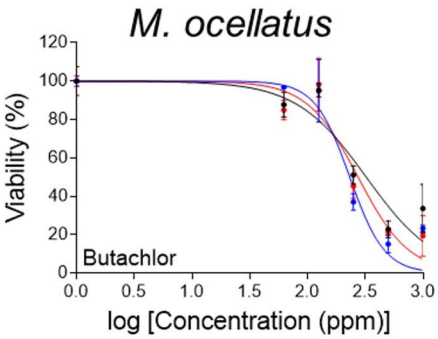
Butachlor	
CAS number	23184-66-9
Molecular structure	
Type	Pesticide
Molecular formula	$C_{17}H_{26}ClNO_2$
Molecular weight	311.8 g/mol

B



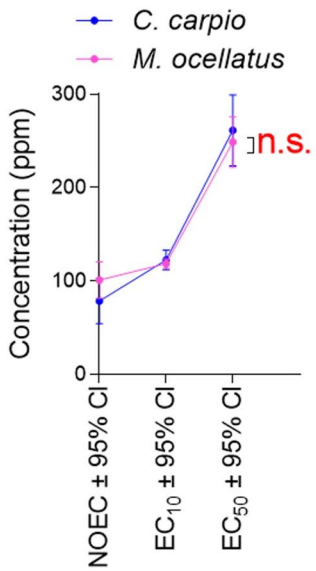
NOEC ± 95% CI (ppm)	EC_{10} ± 95% CI (ppm)	EC_{50} ± 95% CI (ppm)
<78.57 ± 24.131	122.492 ± 10.493	261.423 ± 38.125

C



NOEC ± 95% CI (ppm)	EC_{10} ± 95% CI (ppm)	EC_{50} ± 95% CI (ppm)
<100.996 ± 19.729	118.63 ± 5.044	248.672 ± 27.004

D



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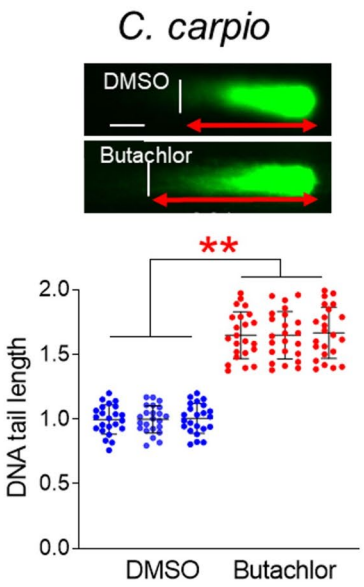


F

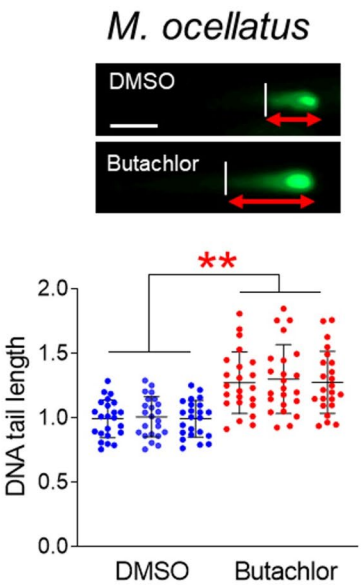


Fig. 1 Comparison of ecotoxicity and genotoxicity of butachlor using *C. carpio* and *M. ocellatus* cells. **a** The CAS number, molecular weight, type, molecular formula, and molecular structure of butachlor are presented. **b**, **c** *C. carpio* and *M. ocellatus* cells were administered with different concentrations of butachlor (0, 62.5, 125, 250, 500, and 1000 ppm) for 6 h in medium containing 1% FBS. Then, cell viability was assessed. For each condition, the experiment was repeated three times, resulting in three independent biological data points (biological triplicates). Each of biological triplicates was derived from analyzing 3 different samples. Furthermore, biological triplicates were reported using mean and 95% confidence interval (CI). The NOEC, EC_{10}, EC_{50}, and 95% CI were calculated using GraphPad Prism 9.0. Table S1 and S2 present the raw data used for the ecotoxicological test of butachlor using *C. carpio* and *M. ocellatus* cells, respectively. **d** Comparison of NOEC, EC_{10}, and EC_{50} values between *C. carpio* and *M. ocellatus* cells. n.s. (not significant), two-way ANOVA followed by Bonferroni post–hoc test. mean ± standard deviation, $n = 3$. **e**, **f** *C. carpio* and *M. ocellatus* cells were administered with butachlor at half the EC_{50}, respectively, for 6 h in medium containing 1% FBS. Then, neutral comet assays were performed. For each condition, the experiment was repeated three times, resulting in three independent biological data points (biological replicates). Each of biological triplicates was derived from analyzing 23 different samples. Furthermore, each of biological triplicates was reported using mean and standard deviation. $^{**}p < 0.01$ for two-way ANOVA followed by Bonferroni post–hoc test. Tables S3 and S4 present the raw data used for the genotoxicity assessment of butachlor using *C. carpio* and *M. ocellatus* cells, respectively

Table 1 Results of ecotoxicological and genotoxicity evaluation using *M. ocellatus* cells (mean ± 95% CI)

Toxicants	Ecotoxicity test			Genotoxicity test (Increase in comet tail compared to the DMSO control)
	NOEC (ppm)	EC_{10} (ppm)	EC_{50} (ppm)	
Butachlor	< 100.996 ± 19.729	118.63 ± 5.044	248.672 ± 27.004	28.5% increase
2,4,6-trichlorophenol	< 102.826 ± 13.135	165.423 ± 21.864	775.231 ± 7.622	7.7% increase
Roxithromycin	< 24.656 ± 15.310	49.311 ± 30.620	703.061 ± 36.393	42.4% increase
Fluxapyroxad	< 157.015 ± 43.056	145.246 ± 40.785	203.8 ± 5.825	54.8% increase
Fipronil	< 11.985 ± 0.992	23.971 ± 1.984	140.51 ± 14.636	25.3% increase
Clarithromycin	< 170.004 ± 45.641	126.667 ± 7.802	507.575 ± 29.619	39.6% increase
2,4-Di-tert-butylphenol	< 49.118 ± 0.668	98.237 ± 1.336	491.186 ± 6.684	245.1% increase
Perfluorooctanoic acid	< 76.009 ± 1.98	152.019 ± 3.961	760.098 ± 19.809	112.0% increase
Prochloraz	< 66.192 ± 12.842	165.477 ± 32.063	475.919 ± 58.720	53.9% increase
Abamectin	< 53.599 ± 54.627	107.197 ± 109.254	242.391 ± 81.801	56.8% increase
Climbazole	< 30.078 ± 11.348	60.155 ± 22.696	242.588 ± 15.258	23.1% increase

using *M. ocellatus* cells were similar to those of the ecotoxicological evaluation using *C. carpio* cells (Fig. 2D).

We then verified whether the genotoxicity evaluation using *M. ocellatus* cells showed similar results to the evaluation using *C. carpio* cells. For a control group, *C. carpio* cells were administered with 2,4,6–trichlorophenol at 410.942 ppm (half of the EC_{50}). In the genotoxicity evaluation using *C. carpio* cells, the comet tail length of 2,4,6–trichlorophenol–treated cells increased by 19.6% compared to the DMSO control (Fig. 2E). For an experimental group, *M. ocellatus* cells were administered with 2,4,6–trichlorophenol at 387.616 ppm (half of the EC_{50}). In the genotoxicity evaluation using *M. ocellatus* cells, the comet tail length of 2,4,6–trichlorophenol–treated cells increased by 7.7% compared to the DMSO control (Fig. 2F and Table 1). These results suggest that genotoxicity assessment using *M. ocellatus* cells responds well to genotoxicity as does genotoxicity assessment using *C. carpio* cells.

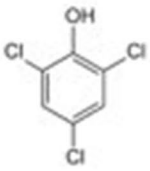
Comparison of ecotoxicity and genotoxicity of roxithromycin using *C. carpio* and *M. ocellatus* cells

Roxithromycin (CAS number: 80214-83-1; $C_{41}H_{76}N_2O_{15}$; molecular weight: 837.05 g/mol) has been used to treat infections of the soft tissues, urinary tract, and respiratory tract (Fig. 3A). This substance can have negative effects on aquatic life. In particular, it can disrupt the reproduction, growth, and antioxidant systems of organisms such as *Daphnia magna*.

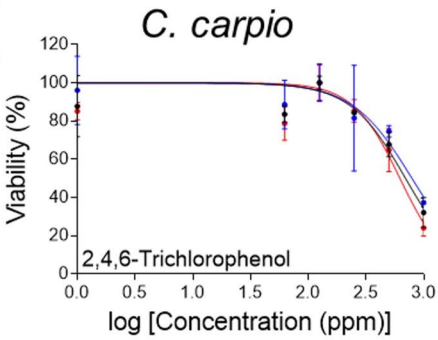
We verified whether the ecotoxicological evaluation using *M. ocellatus* cells showed similar results to the evaluation using *C. carpio* cells. As a control, *C. carpio* cells were treated with various concentrations of roxithromycin (0, 62.5, 125, 250, 500, and 1000 ppm) for 6 h in the medium containing 1% FBS. The effect of roxithromycin on cell viability showed a sigmoid pattern, and the EC_{50} value was 692.975 ± 4.548 (mean ± 95% CI) ppm (Fig. 3B). The NOEC of roxithromycin was less than 42.132 ± 11.094 (mean ± 95% CI) ppm, and the EC_{10} was 103.056 ± 14.037 (mean ± 95% CI) ppm, respectively (Fig. 3B). As an experimental group, *M. ocellatus* cells were treated with various concentrations of roxithromycin (0, 62.5, 125, 250, 500, and 1000 ppm) in a medium containing 1% FBS for 6 h. The effect of roxithromycin on cell viability showed a sigmoid pattern, and the EC_{50} value was 775.231 ± 7.622 (mean ± 95% CI) ppm (Fig. 3C and Table 1). The NOEC of roxithromycin was less than 24.656 ± 15.310 (mean ± 95% CI) ppm, and the EC_{10} was 49.311 ± 30.620 (mean ± 95% CI) ppm (Fig. 3C and Table 1). The NOEC, EC_{10}, and EC_{50} values roxithromycin in *M. ocellatus* cells were very similar to those in *C. carpio* cells, suggesting that the results of the ecotoxicological evaluation using *M. ocellatus* cells were similar to those of the ecotoxicological evaluation using *C. carpio* cells (Fig. 3D).

We then verified whether the genotoxicity evaluation using *M. ocellatus* cells showed similar results to the evaluation using *C. carpio* cells. For a control group, *C. carpio* cells were administered with roxithromycin at 346.488 ppm (half of the EC_{50}). In the genotoxicity evaluation using *C. carpio* cells, the comet tail length of roxithromycin–treated cells increased by 30.9% compared to the DMSO control (Fig. 3E). For an experimental group, *M. ocellatus* cells were administered with roxithromycin at 351.531 ppm (half of the EC_{50}). In the genotoxicity evaluation using *M. ocellatus* cells, the comet tail length of roxithromycin–treated cells increased by 42.4% compared to the DMSO control (Fig. 3F and Table 1). These results suggest that genotoxicity assessment using *M. ocellatus* cells responds well to genotoxicity as does genotoxicity assessment using *C. carpio* cells.

A

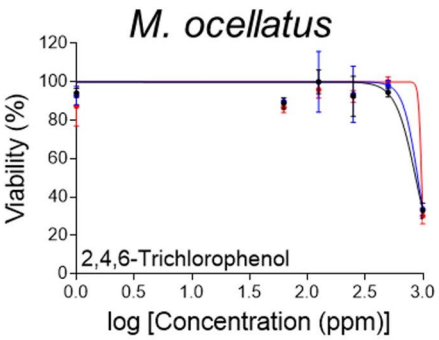
2,4,6–trichlorophenol	
CAS number	88-06-2
Molecular structure	
Type	Phenol
Molecular formula	$C_6H_2Cl_3OH$
Molecular weight	197.4 g/mol

B



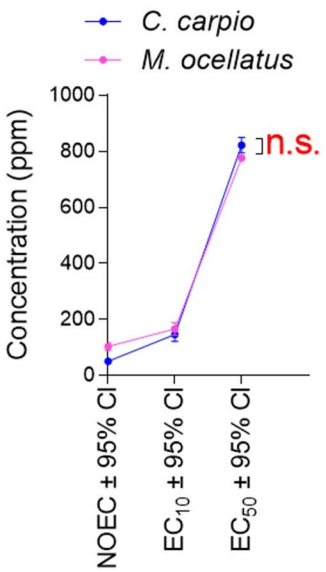
NOEC ± 95% CI (ppm)	EC_{10} ± 95% CI (ppm)	EC_{50} ± 95% CI (ppm)
<49.77 ± 9.976	145.897 ± 24.524	821.884 ± 28.128

C



NOEC ± 9 5% CI (ppm)	EC_{10} ± 95% CI (ppm)	EC_{50} ± 95% CI (ppm)
<102.826 ± 13.135	165.423 ± 21.864	775.231 ± 7.622

D



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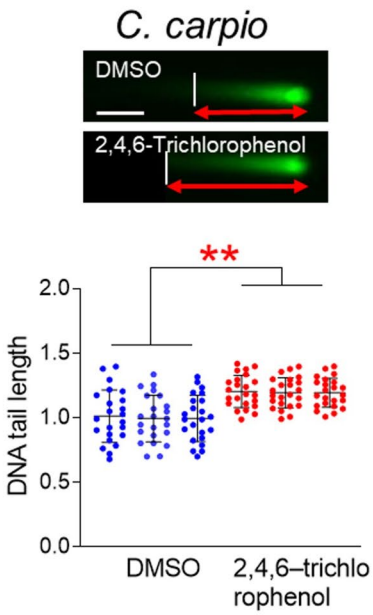

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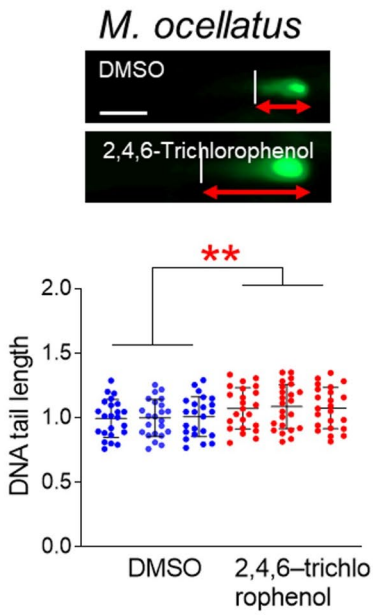


Ecotoxicity and genotoxicity evaluation of fluxapyroxad using *M. ocellatus* cells

The finding that the results of ecotoxicity and genotoxicity assessments using *M. ocellatus cells* were similar to those assessed using *C. carpio* cells allowed us to validate the suitability of the new platform for compounds previously known to be genotoxic.

Fluxapyroxad (CAS number: 907204–31–3; $C_{18}H_{12}F_5N_3O$; molecular weight: 381.3 g/mol) is a succinate dehydrogenase inhibitor and an effective fungicide that kills plant fungal pathogens. Fluxapyroxad can enter aquatic environments through drift, sedimentation, and surface runoff, where it can potentially cause harm to aquatic ecosystems and the environment (Fig. 4A) [22].

Ecotoxicity assessment using 1% FBS/6 h parameter was performed by exposing *M. ocellatus* cells to different doses of fluxapyroxad (0, 62.5, 125, 250, 500, 1000 ppm). The effect of fluxapyroxad on cell viability showed a sigmoid pattern, and the EC_{50} value was 203.8 ± 5.825 (mean ± 95%

◂**Fig. 2** Comparison of ecotoxicity and genotoxicity of 2,4,6–trichlorophenol using *C. carpio* and *M. ocellatus* cells. **a** The CAS number, molecular weight, type, molecular formula, and molecular structure of 2,4,6–trichlorophenol are presented. **b**, **c** *C. carpio* and *M. ocellatus* cells were administered with different concentrations of 2,4,6–trichlorophenol (0, 62.5, 125, 250, 500, and 1000 ppm) for 6 h in medium containing 1% FBS. Then, cell viability was assessed. For each condition, the experiment was repeated three times, resulting in three independent biological data points (biological triplicates). Each of biological triplicates was derived from analyzing 3 different samples. Furthermore, biological triplicates were reported using mean and 95% confidence interval (CI). The NOEC, EC_{10}, EC_{50}, and 95% CI were calculated using GraphPad Prism 9.0. Tables S5 and S6 present the raw data used for the ecotoxicological test of 2,4,6–trichlorophenol using *C. carpio* and *M. ocellatus* cells, respectively. **d** Comparison of NOEC, EC_{10}, and EC_{50} values between *C. carpio* and *M. ocellatus* cells. n.s. (not significant), two-way ANOVA followed by Bonferroni post hoc test. mean ± standard deviation, $n = 3$. **e** and **f** *C. carpio* and *M. ocellatus* cells were administered with 2,4,6–trichlorophenol at half the EC_{50}, respectively, for 6 h in medium containing 1% FBS. Then, neutral comet assays were performed. For each condition, the experiment was repeated three times, resulting in three independent biological data points (biological replicates). Each of biological triplicates was derived from analyzing 23 different samples. Furthermore, each of biological triplicates was reported using mean and standard deviation. $^{**}p < 0.01$ for two-way ANOVA followed by Bonferroni post–hoc test. Tables S7 and S8 present the raw data used for the genotoxicity assessment of 2,4,6–trichlorophenol using *C. carpio* and *M. ocellatus* cells, respectively

CI) ppm (Fig. 4B and Table 1). The NOEC was less than 157.015 ± 43.056 (mean ± 95% CI) ppm, and the EC_{10} was 145.246 ± 40.785 (mean ± 95% CI) ppm (Fig. 4B and Table 1).

Genotoxicity assessment using the 1% FBS/6 h condition was performed at 101.9 ppm (half of the EC_{50}). The comet tail length of fluxapyroxad-treated cells was increased by 54.9% compared with the DMSO control (Fig. 4C and Table 1). These results indicate that fluxapyroxad is genotoxic and should be used with extreme caution.

Ecotoxicity and genotoxicity evaluation of fipronil using *M. ocellatus* cells

Fipronil (CAS number: 120068–37–3; $C_{12}H_4Cl_2F_6N_4OS$; molecular weight: 437.1 g/mol) is a phenylpyrazole insecticide used in veterinary medicine and agriculture (Fig. 5A) [23]. Fipronil is classified as a carcinogen by the US Environmental Protection Agency and has been reported to be highly toxic to aquatic invertebrates and fish [23].

Ecotoxicity assessment using the 1% FBS/6 h condition was performed by exposing *M. ocellatus* cells to different doses of fipronil (0, 62.5, 125, 250, 500, 1000 ppm). The effect of fipronil on cell viability showed a sigmoid pattern, and the EC_{50} value was 140.51 ± 14.636 (mean ± 95% CI) ppm (Fig. 5B and Table 1). The NOEC was less than 11.985 ± 0.992 (mean ± 95% CI) ppm, and the EC_{10} was 23.971 ± 1.984 (mean ± 95% CI) ppm (Fig. 5B and Table 1).

Genotoxicity assessment using 1% FBS/6 h parameter was performed at 70.255 ppm (half of the EC_{50}). The comet tail length of fipronil-treated cells was increased by 25.3% compared to the DMSO control, indicating the genotoxicity of fipronil (Fig. 5C and Table 1).

Ecotoxicity and genotoxicity evaluation of clarithromycin using *M. ocellatus* cells

Clarithromycin (CAS number: 81103–11-9; $C_{38}H_{69}NO_{13}$; molecular weight: 748.0 g/mol) is a widely used antibiotic with a long half-life and high antibacterial activity (Fig. 6A) [24]. However, its low biodegradability promotes antimicrobial resistance in bacteria and adversely affects aquatic organisms, posing a serious risk to the aquatic environment [24].

Ecotoxicity assessment using the 1% FBS/6 h condition was performed by exposing *M. ocellatus* cells to different doses of clarithromycin (0, 62.5, 125, 250, 500, 1000 ppm). The effect of clarithromycin on cell viability showed a sigmoid pattern, and the EC_{50} value was 507.575 ± 29.619 (mean ± 95% CI) ppm (Fig. 6B and Table 1). The NOEC was less than 170.004 ± 45.641 (mean ± 95% CI) ppm, and the EC_{10} was 126.667 ± 7.802 (mean ± 95% CI) ppm (Fig. 6B and Table 1).

Genotoxicity assessment using the 1% FBS/6 h condition was performed at 253.7875 ppm (half of the EC_{50}). The comet tail length of clarithromycin-treated cells was increased by 39.6% compared to the DMSO control, indicating the genotoxicity of clarithromycin (Fig. 6C and Table 1).

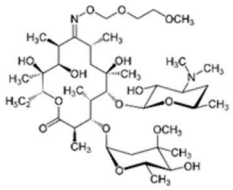
Ecotoxicity and genotoxicity evaluation of 2,4-Di-tert-butylphenol using *M. ocellatus* cells

2,4-Di-tert-butylphenol (CAS number: 96–76-4; $C_{14}H_{22}O$; molecular weight: 206.32 g/mol) is a commonly used additive to improve the performance and durability of plastic materials (Fig. 7A) [25]. 2,4-Di-tert-butylphenol is released in high concentrations from plastic waste, masks, and pollutants in the marine environment, and is easily accumulated in aquatic organisms [26].

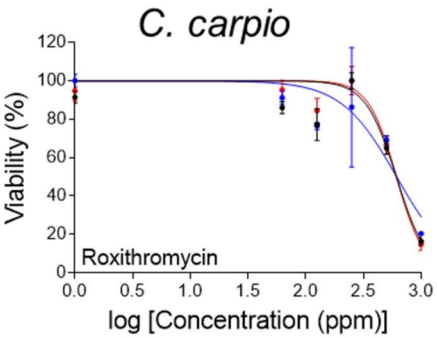
Ecotoxicity assessment using the 1% FBS/6 h condition was performed by exposing *M. ocellatus* cells to various doses of 2,4–Di–tert–butylphenol (0, 62.5, 125, 250, 500, 1000 ppm). The effect of 2,4-Di-tert-butylphenol on cell viability showed a linear pattern, and the EC_{50} value was 491.186 ± 6.684 (mean ± 95% CI) ppm (Fig. 7B and Table 1). The NOEC was less than 49.118 ± 0.668 (mean ± 95% CI) ppm, and the EC_{10} was 98.237 ± 1.336 (mean ± 95% CI) ppm (Fig. 7B and Table 1).

Genotoxicity assessment using the 1% FBS/6 h condition was performed at 245.593 ppm (half of the EC_{50}). The comet tail length of clarithromycin-treated cells was increased

A

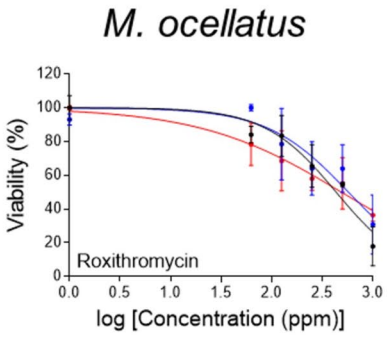
Roxithromycin	
CAS number	80214-83-1
Molecular structure	
Type	Medicine
Molecular formula	$C_{41}H_{76}N_2O_{15}$
Molecular weight	837.05 g/mol

B



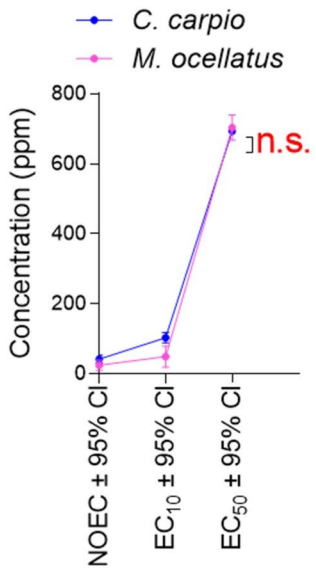
NOEC ± 95% CI (ppm)	EC_{10} ± 95% CI (ppm)	EC_{50} ± 95% CI (ppm)
<42.132 ± 11.094	103.056 ± 14.037	692.975 ± 4.548

C

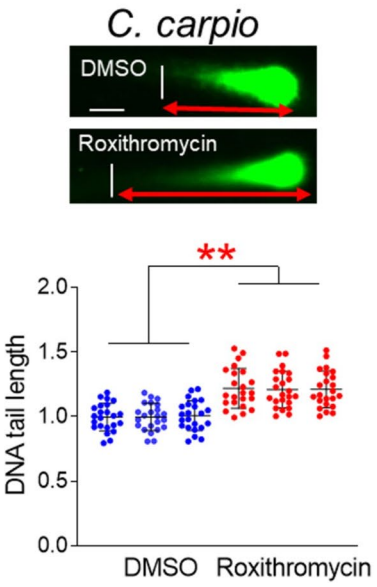


NOEC ± 9 5% CI (ppm)	EC_{10} ± 95% CI (ppm)	EC_{50} ± 95% CI (ppm)
<24.656 ± 15.310	49.311 ± 30.620	703.061 ± 36.393

D



E



F

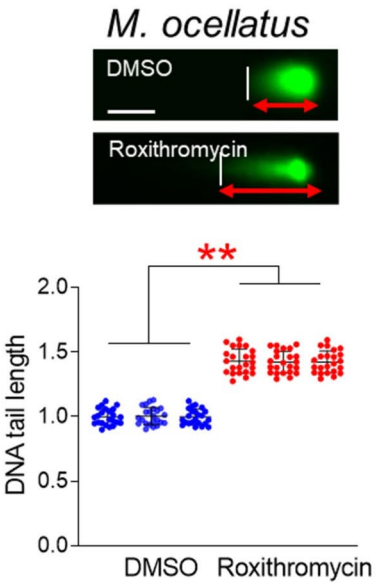


Fig. 3 Comparison of ecotoxicity and genotoxicity of roxithromycin using *C. carpio* and *M. ocellatus* cells. **a** The CAS number, molecular weight, type, molecular formula, and molecular structure of roxithromycin are presented. **b**, **c** *C. carpio* and *M. ocellatus* cells were administered with different concentrations of roxithromycin (0, 62.5, 125, 250, 500, and 1000 ppm) for 6 h in medium containing 1% FBS. Then, cell viability was assessed. For each condition, the experiment was repeated three times, resulting in three independent biological data points (biological triplicates). Each of biological triplicates was derived from analyzing 3 different samples. Furthermore, biological triplicates were reported using mean and 95% confidence interval (CI). The NOEC, EC_{10}, EC_{50}, and 95% CI were calculated using GraphPad Prism 9.0. Tables S9 and S10 present the raw data used for the ecotoxicological test of roxithromycin using *C. carpio* and *M. ocellatus* cells, respectively. **d** Comparison of NOEC, EC_{10}, and EC_{50} values between *C. carpio* and *M. ocellatus* cells. n.s. (not significant), two-way ANOVA followed by Bonferroni post hoc test. mean ± standard deviation, $n = 3$. **e**, **f** *C. carpio* and *M. ocellatus* cells were administered with roxithromycin at half the EC_{50}, respectively, for 6 h in medium containing 1% FBS. Then, neutral comet assays were performed. For each condition, the experiment was repeated three times, resulting in three independent biological data points (biological replicates). Each of biological triplicates was derived from analyzing 23 different samples. Furthermore, each of biological triplicates was reported using mean and standard deviation. $^{**}p < 0.01$ for two-way ANOVA followed by Bonferroni post–hoc test. Tables S11 and 12 present the raw data used for the genotoxicity assessment of roxithromycin using *C. carpio* and *M. ocellatus* cells, respectively

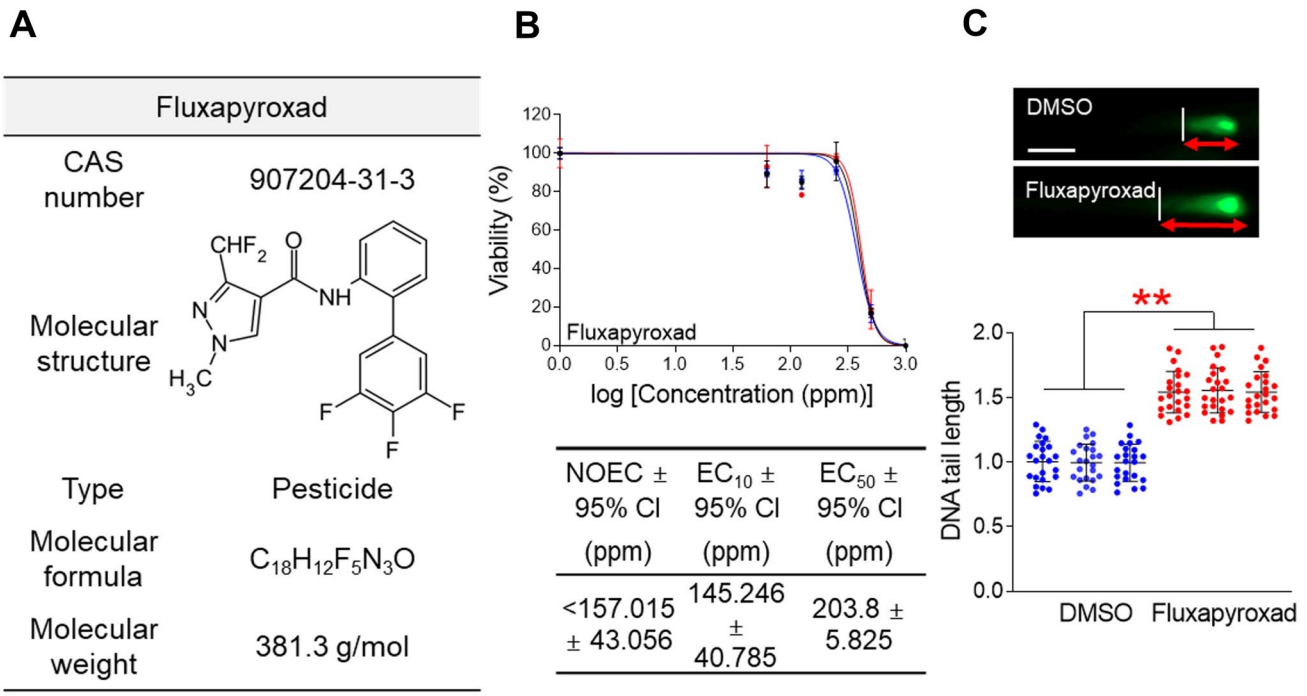


Fig. 4 Ecotoxicity and genotoxicity evaluation of fluxapyroxad using *M. ocellatus* cells. **a** The CAS number, molecular weight, type, molecular formula, and molecular structure of fluxapyroxad are presented. **b** *M. ocellatus* cells were administered with different concentrations of fluxapyroxad (0, 62.5, 125, 250, 500, and 1000 ppm) for 6 h in medium containing 1% FBS. Then, cell viability was assessed. For each condition, the experiment was repeated three times, resulting in three independent biological data points (biological triplicates). Each of biological triplicates was derived from analyzing 3 different samples. Furthermore, biological triplicates were reported using mean and 95% confidence interval (CI). The NOEC, EC_{10}, EC_{50}, and 95% CI were calculated using GraphPad Prism 9.0. Table S13 presents the raw data used for the ecotoxicological test of fluxapyroxad using *M. ocellatus* cells. **c** *M. ocellatus* cells were administered with fluxapyroxad at half the EC_{50} for 6 h in medium containing 1% FBS. Then, neutral comet assays were performed. For each condition, the experiment was repeated three times, resulting in three independent biological data points (biological replicates). Each of biological triplicates was derived from analyzing 23 different samples. Furthermore, each of biological triplicates was reported using mean and standard deviation. $^{**}p < 0.01$ for two-way ANOVA followed by Bonferroni post–hoc test. Table S14 presents the raw data used for the genotoxicity assessment of fluxapyroxad using *M. ocellatus* cells

by 245.10% compared to the DMSO control (Fig. 7C and Table 1). These data suggest that 2,4-Di-tert-butylphenol should be used with caution because it is added to plastics used in everyday life.

Ecotoxicity and genotoxicity evaluation of perfluorooctanoic acid using *M. ocellatus* cells

Perfluorooctanoic acid (CAS number: 335-67-1; $C_8HF_{15}O_2$; molecular weight: 414.07 g/mol) is a fluorinated organic compound that has been widely used in various industries because of its surfactant and anti-wetting properties (Fig. 8A) [27]. Perfluorooctanoic acid is highly resistant to biological degradation, degrades very slowly, and persists in the environment for a long time, so it has been classified as a persistent chemical and its use has been banned in many countries [28]. In addition, long-term presence in the environment acts as a carcinogenic agent, posing a serious risk to human health [29].

Ecotoxicity assessment using the 1% FBS/6 h condition was performed by exposing *M. ocellatus* cells to different doses of perfluorooctanoic acid (0, 62.5, 125, 250, 500, 1000 ppm). The effect of perfluorooctanoic acid on cell viability showed a sigmoid pattern, and the EC_{50} value was 760.098 ± 19.809 (mean ± 95% CI) ppm (Fig. 8B and Table 1). The NOEC was less than 76.009 ± 1.98 (mean ± 95% CI) ppm, and the EC_{10} was 152.019 ± 3.961 (mean ± 95% CI) ppm (Fig. 8B and Table 1).

Genotoxicity assessment using the 1% FBS/6 h condition was performed at 380.049 ppm (half of the EC_{50}). The comet tail length of perfluorooctanoic acid-treated cells was increased by 112.0% compared to the DMSO control (Fig. 8C and Table 1). These data suggest that caution should be used when using perfluorooctanoic acid, as it persists in the environment for a long time and is a carcinogen.

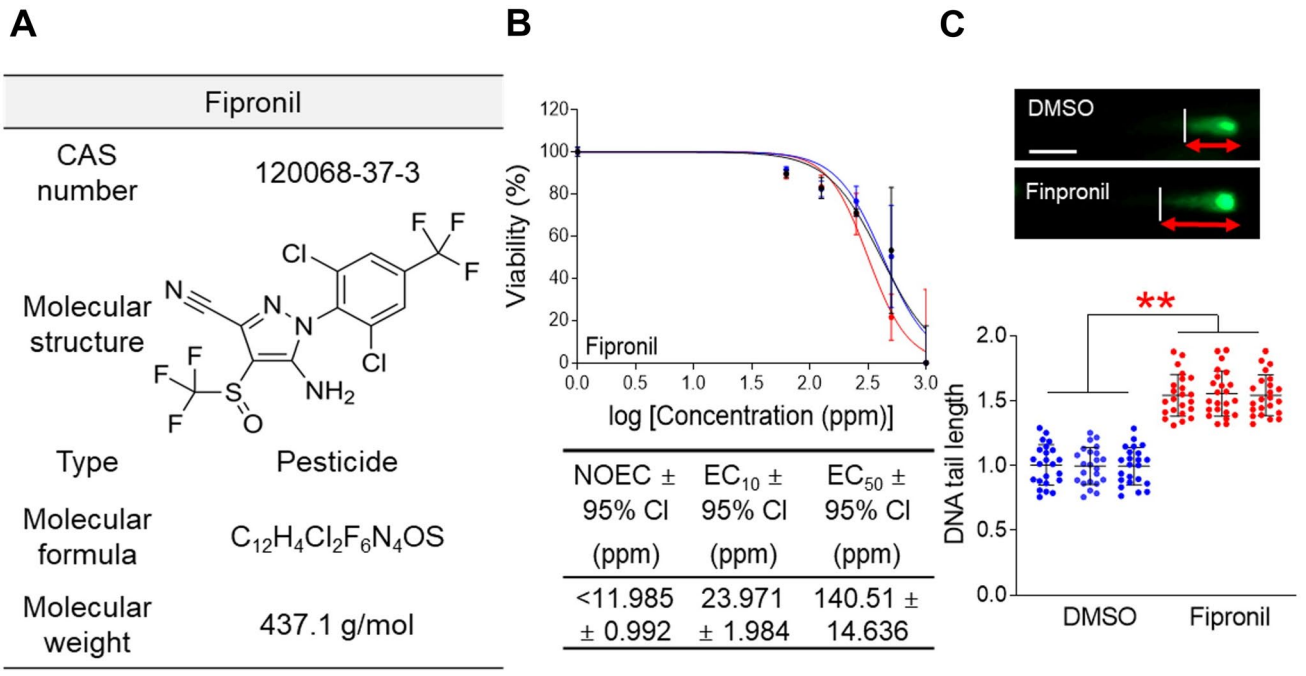


Fig. 5 Ecotoxicity and genotoxicity evaluation of fipronil using *M. ocellatus* cells. **a** The CAS number, molecular weight, type, molecular formula, and molecular structure of fipronil are presented. **b** *M. ocellatus* cells were administered with different concentrations of fipronil (0, 62.5, 125, 250, 500, and 1000 ppm) for 6 h in medium containing 1% FBS. Then, cell viability was assessed. For each condition, the experiment was repeated three times, resulting in three independent biological data points (biological triplicates). Each of biological triplicates was derived from analyzing 3 different samples. Furthermore, biological triplicates were reported using mean and 95% confidence interval (CI). The NOEC, EC_{10}, EC_{50}, and 95% CI were calculated using GraphPad Prism 9.0. Table S15 presents the raw data used for the ecotoxicological test of fipronil using *M. ocellatus* cells. **c** *M. ocellatus* cells were administered with fipronil at half the EC_{50} for 6 h in medium containing 1% FBS. Then, neutral comet assays were performed. For each condition, the experiment was repeated three times, resulting in three independent biological data points (biological replicates). Each of biological triplicates was derived from analyzing 23 different samples. Furthermore, each of biological triplicates was reported using mean and standard deviation. $^{**}p < 0.01$ for two-way ANOVA followed by Bonferroni post–hoc test. Table S16 presents the raw data used for the genotoxicity assessment of fipronil using *M. ocellatus* cells

Ecotoxicity and genotoxicity evaluation of prochloraz acid using *M. ocellatus* cells

Prochloraz acid (CAS number: 67747–09-5; $C_{15}H_{16}Cl_3N_3O_2$; molecular weight: 376.67 g/mol) is a fungicide, which specifically targets and controls fungal growth [30] (Fig. 9A). Prochloraz acid's chemical structure, particularly the imidazole ring and the trichlorophenol group is highly resistant to biological degradation, thereby degrading very slowly and persisting for a long time [31]. In addition, it is classified as a Group C carcinogen, which is considered a possible carcinogen for humans [32].

Ecotoxicity assessment using the 1% FBS/6 h condition was performed by exposing *M. ocellatus* cells to different doses of prochloraz acid (0, 62.5, 125, 250, 500, 1000 ppm). The effect of prochloraz acid on cell viability showed a sigmoid pattern, and the EC_{50} value was 475.919 ± 58.720 (mean ± 95% CI) ppm (Fig. 9B and Table 1). The NOEC was less than 66.192 ± 12.842 (mean ± 95% CI) ppm, and the EC_{10} was 66.192 ± 12.842 (mean ± 95% CI) ppm (Fig. 9B and Table 1).

Genotoxicity assessment using the 1% FBS/6 h condition was performed at 237.960 ppm (half of the EC_{50}). The comet tail length of prochloraz acid-treated cells was increased by 53.9% compared to the DMSO control (Fig. 9C and Table 1). These data suggest that caution should be used when using prochloraz acid, as it persists in the environment for a long time and is a carcinogen.

Ecotoxicity and genotoxicity evaluation of abamectin using *M. ocellatus* cells

Abamectin (CAS number 71751–41-2; $C_{95}H_{142}O_{28}$; molecular weight: 1,732.1 g/mol) is a member of the avermectin family and acts as an insecticide by paralyzing insects and mites [33] (Fig. 10A). Abamectin degrades relatively quickly in water, but can persist in surface waters due to agricultural runoff [34]. Residual abamectin

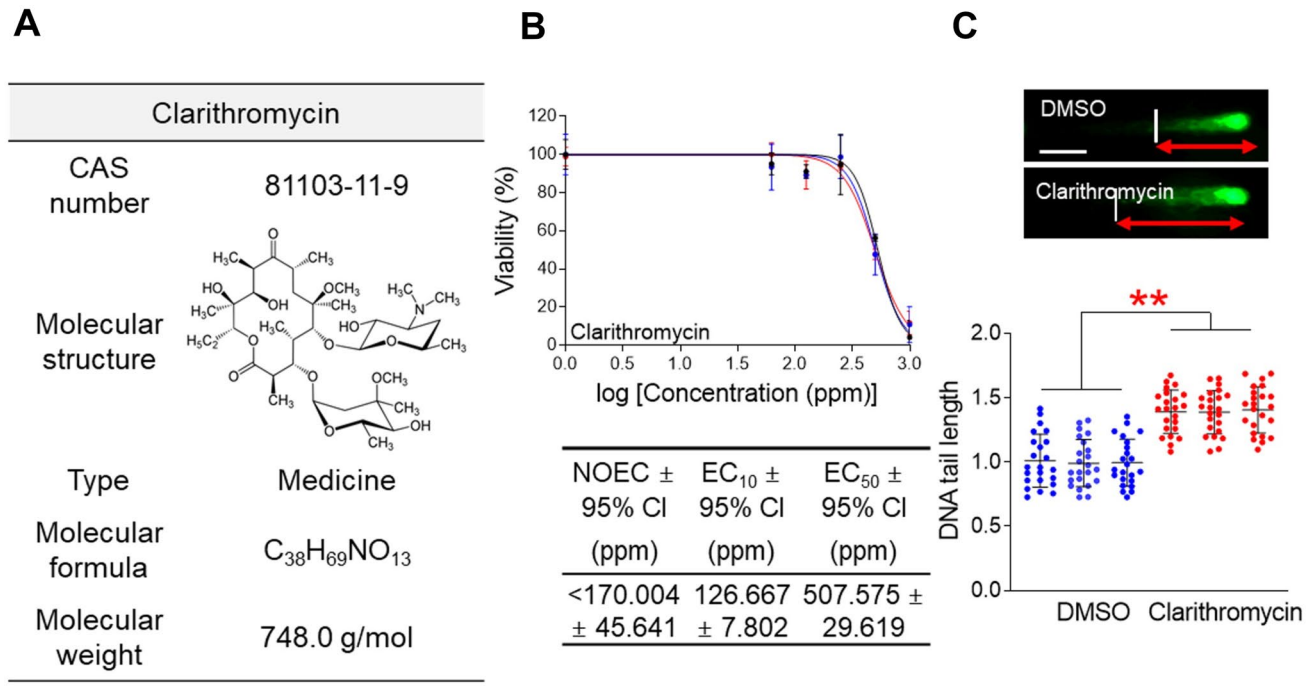


Fig. 6 Ecotoxicity and genotoxicity evaluation of clarithromycin using *M. ocellatus* cells. **a** The CAS number, molecular weight, type, molecular formula, and molecular structure of clarithromycin are presented. **b** *M. ocellatus* cells were administered with different concentrations of clarithromycin (0, 62.5, 125, 250, 500, and 1000 ppm) for 6 h in medium containing 1% FBS. Then, cell viability was assessed. For each condition, the experiment was repeated three times, resulting in three independent biological data points (biological triplicates). Each of biological triplicates was derived from analyzing 3 different samples. Furthermore, biological triplicates were reported using mean and 95% confidence interval (CI). The NOEC, EC_{10}, EC_{50}, and 95% CI were calculated using GraphPad Prism 9.0. Table S17 presents the raw data used for the ecotoxicological test of clarithromycin using *M. ocellatus* cells. **c** *M. ocellatus* cells were administered with clarithromycin at half the EC_{50} for 6 h in medium containing 1% FBS. Then, neutral comet assays were performed. For each condition, the experiment was repeated three times, resulting in three independent biological data points (biological replicates). Each of biological triplicates was derived from analyzing 23 different samples. Furthermore, each of biological triplicates was reported using mean and standard deviation. $^{**}p < 0.01$ for two-way ANOVA followed by Bonferroni post–hoc test. Table S18 presents the raw data used for the genotoxicity assessment of clarithromycin using *M. ocellatus* cells

poses a serious risk to aquatic ecosystems, requiring strict management of its use.

Ecotoxicity assessment using the 1% FBS/6 h condition was performed by exposing *M. ocellatus* cells to different doses of abamectin (0, 62.5, 125, 250, 500, 1000 ppm). The effect of abamectin on cell viability showed a sigmoid pattern, and the EC_{50} value was 242.391 ± 81.801 (mean ± 95% CI) ppm (Fig. 10B and Table 1). The NOEC was less than 53.599 ± 54.627 (mean ± 95% CI) ppm, and the EC_{10} was 107.197 ± 109.254 (mean ± 95% CI) ppm (Fig. 10B and Table 1).

Genotoxicity assessment using the 1% FBS/6 h condition was performed at 121.196 ppm (half of the EC_{50}). The comet tail length of abamectin-treated cells was increased by 56.8% compared to the DMSO control (Fig. 10C and Table 1). These data suggest that caution should be used when using abamectin.

Ecotoxicity and genotoxicity evaluation of climbazole using *M. ocellatus* cells

Climbazole (CAS number 38083-17-9; C15H17ClN2O2; molecular weight: 292.76 g/mol), a common antifungal agent, works by blocking enzymes that produce sterols and altering the function of the fungal cell membrane [35] (Fig. 11A). Climbazole, which has antifungal properties, is commonly found in shampoos and is considered one of the major causes of aquatic contamination [36].

Ecotoxicity assessment using the 1% FBS/6 h condition was performed by exposing *M. ocellatus* cells to different doses of climbazole (0, 62.5, 125, 250, 500, 1000 ppm). The effect of climbazole on cell viability showed a sigmoid pattern, and the EC_{50} value was 242.588 ± 15.258 (mean ± 95% CI) ppm (Fig. 11B and Table 1). The NOEC was less than 30.078 ± 11.348 (mean ± 95% CI) ppm,

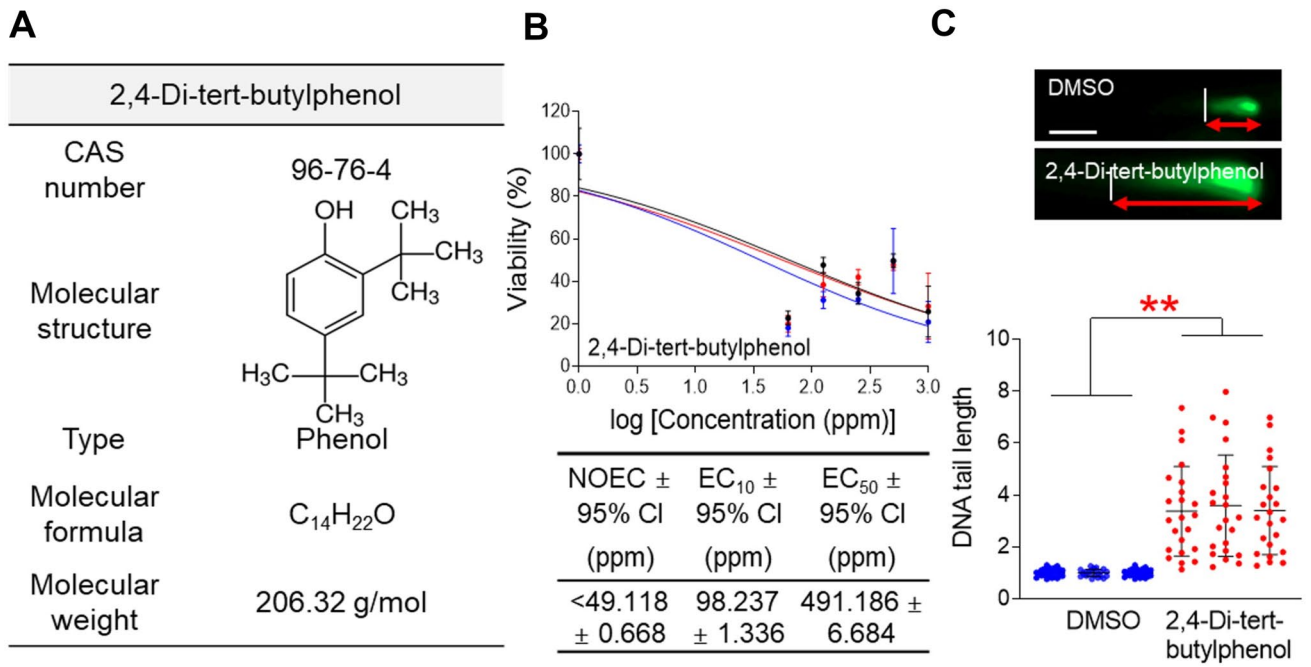


Fig. 7 Ecotoxicity and genotoxicity evaluation of 2,4-Di-tert-butylphenol using *M. ocellatus* cells. **a** The CAS number, molecular weight, type, molecular formula, and molecular structure of 2,4-Di-tert-butylphenol are presented. **b** *M. ocellatus* cells were administered with different concentrations of 2,4-Di-tert-butylphenol (0, 62.5, 125, 250, 500, and 1000 ppm) for 6 h in medium containing 1% FBS. Then, cell viability was assessed. For each condition, the experiment was repeated three times, resulting in three independent biological data points (biological triplicates). Each of biological triplicates was derived from analyzing 3 different samples. Furthermore, biological triplicates were reported using mean and 95% confidence interval (CI). The NOEC, EC_{10}, EC_{50}, and 95% CI were calculated using GraphPad Prism 9.0. Table S19 presents the raw data used for the ecotoxicological test of 2,4-Di-tert-butylphenol using *M. ocellatus* cells. **c** *M. ocellatus* cells were administered with 2,4-Di-tert-butylphenol at half the EC_{50} dose for 6 h in medium containing 1% FBS. Then, neutral comet assays were performed. For each condition, the experiment was repeated three times, resulting in three independent biological data points (biological replicates). Each of biological triplicates was derived from analyzing 23 different samples. Furthermore, each of biological triplicates was reported using mean and standard deviation. $^{**}p < 0.01$ for two-way ANOVA followed by Bonferroni post–hoc test. Table S20 presents the raw data used for the genotoxicity assessment of 2,4-Di-tert-butylphenol using *M. ocellatus* cells

and the EC_{10} was 60.155 ± 22.696 (mean ± 95% CI) ppm (Fig. 11B and Table 1).

Genotoxicity assessment using the 1% FBS/6 h condition was performed at 121.294 ppm (half of the EC_{50}). The comet tail length of climbazole-treated cells was increased by 23.1% compared to the DMSO control (Fig. 11C and Table 1). These data suggest that genotoxic climbazole should be used with caution.

Discussion

Aquatic ecosystems are under threat from toxicants released from nearby farms and factories [37]. Fish cell-based ecotoxicity and genotoxicity assessments are valuable methods for assessing toxicants and have been used as useful tools for protecting ecosystems [38]. Fish cells provide consistent testing conditions without variability due to fish behavior [39]. Moreover, fish cells are sensitive to toxicants and respond to lower doses of toxicants [9]. Furthermore, using fish cells from the same individual for both control and experimental groups minimizes genetic variation within the assay and allows precise control of environmental factors such as pH and temperature, generating highly reproducible data [40]. Therefore, fish cells have become an indispensable assessment tool for ecotoxicity and genotoxicity assessments. However, there are areas where improvements are needed in the assessment methods using fish cells. These assessment methods require toxicity treatment for 1 to 36 days, making them unsuitable for situations requiring rapid toxicity assessment [4]. Recently, a platform for measuring ecotoxicity and genotoxicity using *C. carpio* cells for only 6 h was developed [14, 15]. This new ecotoxicity and genotoxicity method provided data more quickly than the existing evaluation methods [14, 15]. However, whether applying these two parameters to cells derived from other fish species would yield fast and accurate data has not been studied. In this study, the two parameters were applied to cells derived from *M. ocellatus* fish. The ecotoxicological data using *M. ocellatus* cells were similar to those using *C.*

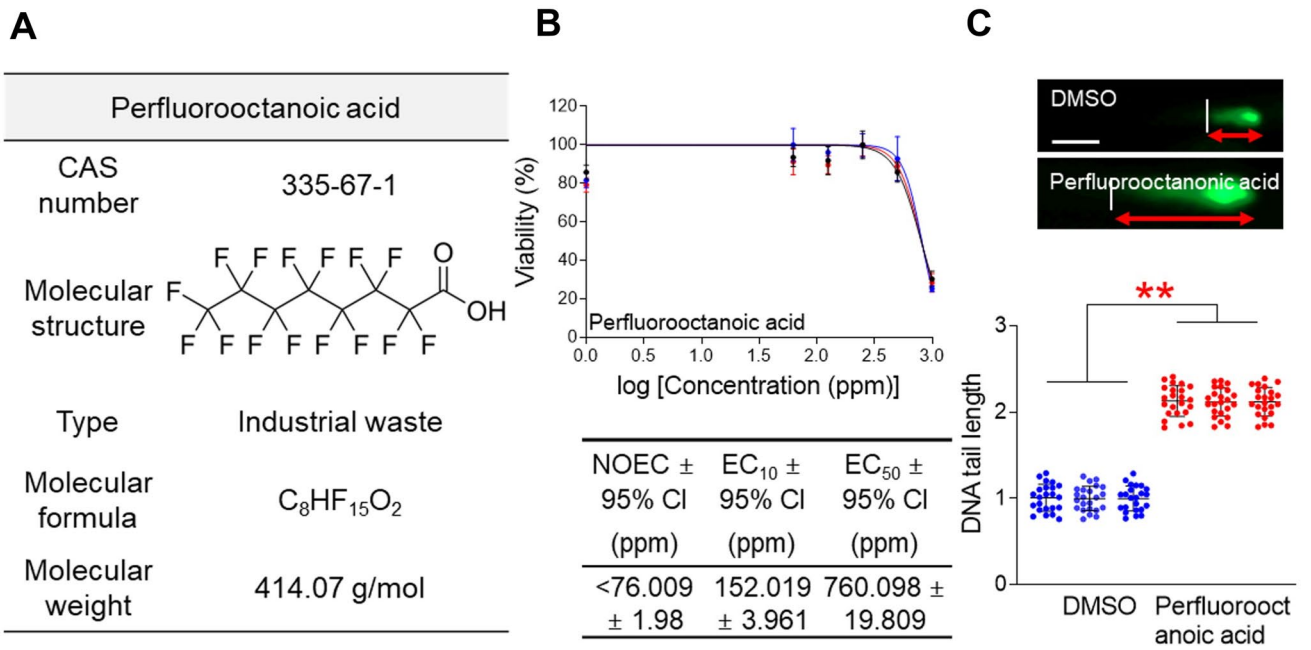


Fig. 8 Ecotoxicity and genotoxicity evaluation of perfluorooctanoic acid using *M. ocellatus* cells. **a** The CAS number, molecular weight, type, molecular formula, and molecular structure of perfluorooctanoic acid are presented. **b** *M. ocellatus* cells were administered with different concentrations of perfluorooctanoic acid (0, 62.5, 125, 250, 500, and 1000 ppm) for 6 h in medium containing 1% FBS. Then, cell viability was assessed. For each condition, the experiment was repeated three times, resulting in three independent biological data points (biological triplicates). Each of biological triplicates was derived from analyzing 3 different samples. Furthermore, biological triplicates were reported using mean and 95% confidence interval (CI). The NOEC, EC_{10}, EC_{50}, and 95% CI were calculated using GraphPad Prism 9.0. Table S21 presents the raw data used for the ecotoxicological test of perfluorooctanoic acid using *M. ocellatus* cells. **c** *M. ocellatus* cells were administered with perfluorooctanoic acid at half the EC_{50} for 6 h in medium containing 1% FBS. Then, neutral comet assays were performed. For each condition, the experiment was repeated three times, resulting in three independent biological data points (biological replicates). Each of biological triplicates was derived from analyzing 23 different samples. Furthermore, each of biological triplicates was reported using mean and standard deviation. $^{**}p < 0.01$ for two-way ANOVA followed by Bonferroni post–hoc test. Table S22 presents the raw data used for the genotoxicity assessment of perfluorooctanoic acid using *M. ocellatus* cells

carpio cells. Moreover, the genotoxicity evaluation using *M. ocellatus* cells significantly increased the size of the comet tail in response to the genotoxin, similar to the genotoxicity evaluation using *C. carpio* cells. This new platform will be a crucial tool for rapid monitoring of toxicants and protecting aquatic ecosystems. However, it was tested only under the 1% FBS/6 h condition, so further studies are needed. It is expected that better conditions can be established by applying various FBS concentrations and treatment periods.

It is estimated that over 350,000 compounds have been registered for commercial production, and new chemicals are continuously being synthesized [41]. Since only a small number of compounds have undergone ecotoxicity or genotoxicity evaluation, there is an urgent need to evaluate the remaining untested compounds [42]. Current ecotoxicity or genotoxicity evaluation technologies take 1–36 days to complete the test [4], which is insufficient to perform the test of the remaining untested compounds. Therefore, a new platform for ecotoxicity and genotoxicity evaluation is needed. The ecotoxicity and genotoxicity evaluation developed in this study can fully meet this need. This platform using *M. ocellatus* cells allows one researcher to evaluate 20–25 substances per day. To evaluate twice as many substances per day, researchers can even start a second testing cycle before completing the first. Consequently, this assessment platform can rapidly collect ecotoxicity and genotoxicity information for a wide range of compounds, suggesting that it may be a useful tool for assessing the toxicity of untested ones.

Industrial wastewaters, even in relatively small quantities, accumulate in the body of aquatic organisms and threaten them [43]. They contain a variety of hazardous substances, including pesticides, pharmaceuticals, phenols, and industrial chemicals [2]. Therefore, it is urgent to develop a universal ecotoxicity or genotoxicity method that can assess a wide range of toxicants present in industrial wastewaters. Here, we evaluated whether the platform is suitable for evaluating ecotoxicity and genotoxicity using industrial wastewater components known to be genotoxic: pesticides (butachlor, fluxapyroxad, fipronil, prochloraz, and abamectin), pharmaceuticals (roxithromycin, clarithromycin, and climbazole), phenols (2,4,6-trichlorophenol and 2,4-di-tert-butylphenol), and industrial chemicals (perfluorooctanoic

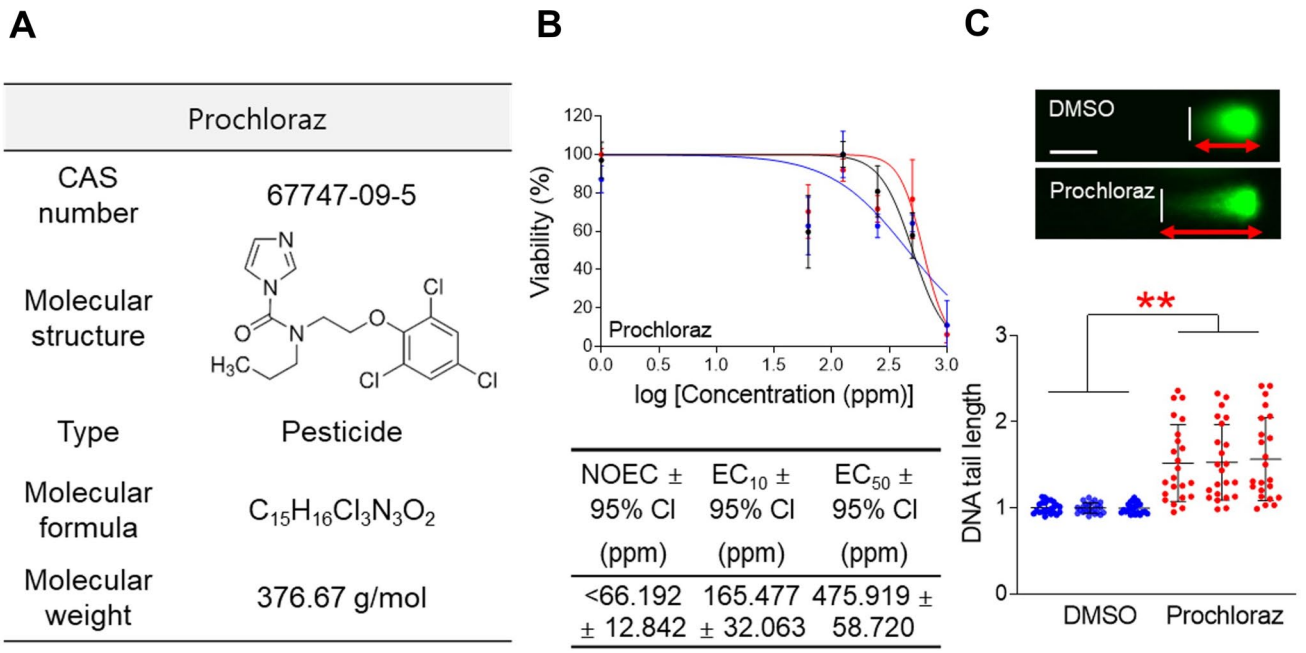
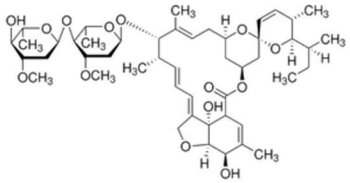


Fig. 9 Ecotoxicity and genotoxicity evaluation of prochloraz using *M. ocellatus* cells. **a** The CAS number, molecular weight, type, molecular formula, and molecular structure of prochloraz are presented. **b** *M. ocellatus* cells were administered with different concentrations of prochloraz (0, 62.5, 125, 250, 500, and 1000 ppm) for 6 h in medium containing 1% FBS. Then, cell viability was assessed. For each condition, the experiment was repeated three times, resulting in three independent biological data points (biological triplicates). Each of biological triplicates was derived from analyzing 3 different samples. Furthermore, biological triplicates were reported using mean and 95% confidence interval (CI). The NOEC, EC_{10}, EC_{50}, and 95% CI were calculated using GraphPad Prism 9.0. Table S23 presents the raw data used for the ecotoxicological test of prochloraz using *M. ocellatus* cells. **c** *M. ocellatus* cells were administered with prochloraz at half the EC_{50} for 6 h in medium containing 1% FBS. Then, neutral comet assays were performed. For each condition, the experiment was repeated three times, resulting in three independent biological data points (biological replicates). Each of biological triplicates was derived from analyzing 23 different samples. Furthermore, each of biological triplicates was reported using mean and standard deviation. $^{**}p < 0.01$ for two-way ANOVA followed by Bonferroni post–hoc test. Table S24 presents the raw data used for the genotoxicity assessment of prochloraz using *M. ocellatus* cells

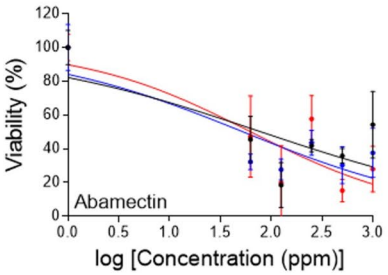
acid). The platform showed consistent ecotoxicity results with low 95% CIs in triple ecotoxicity tests for toxicants, suggesting the universal applicability of the new platform for ecotoxicity assessment. Results of the genotoxicity tests showed that all tested toxicants increased the comet tail length compared to the control, indicating the universal applicability of the new platform for genotoxicity assessment. The ability to detect genotoxicity of pesticides, pharmaceuticals, phenols, and industrial chemicals suggests that the method can be applied to complex chemical compositions commonly found in industrial wastewater. Here, we propose that the integration of this platform into environmental monitoring pipelines will enhance early warning capabilities and provide information for timely regulatory responses.

There were differences in the sensitivity to genotoxins between *M. ocellatus* and *C. carpio* cells. This could be due to differences in sensitivity between cell types, but the data did not support this hypothesis. For example, the genotoxicity of *M. ocellatus* cells to butachlor (comet tail length: 28.5% increase compared to DMSO control) was lower than that of *C. carpio* cells (comet tail length: 65.4% increase compared to DMSO control). Furthermore, the genotoxicity of *M. ocellatus* cells to 2,4,6-trichlorophenol (comet tail length: 7.7% increase compared to DMSO control) was lower than that of *C. carpio* cells (comet tail length: 19.6% increase compared to DMSO control). However, the genotoxicity of roxithromycin to *M. ocellatus* cells (comet tail length: 42.74% increase compared to DMSO control) was higher than that to *C. carpio* cells (comet tail length: 21.5% increase compared to DMSO control). Because *M. ocellatus* cells were more sensitive to roxithromycin than *C. carpio* cells, it is difficult to conclude that *M. ocellatus* cells are less sensitive to genotoxins than *C. carpio* cells. Therefore, we hypothesized that the difference in sensitivity to genotoxins may be due to the concentration of genotoxins, and the data support this hypothesis. Half of the EC_{50} concentration was used for genotoxicity assessment [19, 20]. For butachlor, 124.336 ppm was used in *M. ocellatus* cells, whereas 130.712 ppm was used in *C. carpio* cells. The comet tail length was longer in *C. carpio* cells, which may be because the butachlor concentration used in

A

Abamectin	
CAS number	71751-41-2
Molecular structure	
Type	Pesticide
Molecular formula	$C_{95}H_{142}O_{28}$
Molecular weight	1732.1 g/mol

B



NOEC ± 95 % CI (ppm)	EC_{10} ± 95% CI (ppm)	EC_{50} ± 95% CI (ppm)
<53.599 ± 54.627	107.197 ± 109.254	242.391 ± 81.801

C

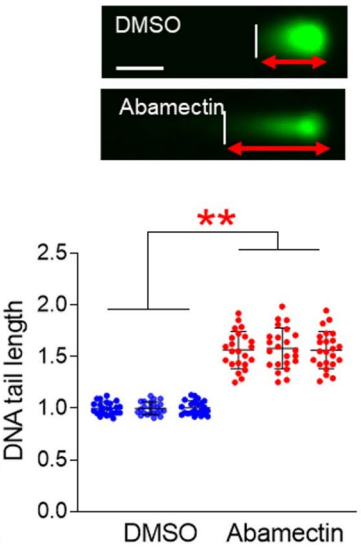


Fig. 10 Ecotoxicity and genotoxicity evaluation of abamectin using *M. ocellatus* cells. **a** The CAS number, molecular weight, type, molecular formula, and molecular structure of abamectin are presented. **b** *M. ocellatus* cells were administered with different concentrations of abamectin (0, 62.5, 125, 250, 500, and 1000 ppm) for 6 h in medium containing 1% FBS. Then, cell viability was assessed. For each condition, the experiment was repeated three times, resulting in three independent biological data points (biological triplicates). Each of biological triplicates was derived from analyzing 3 different samples. Furthermore, biological triplicates were reported using mean and 95% confidence interval (CI). The NOEC, EC_{10}, EC_{50}, and 95% CI were calculated using GraphPad Prism 9.0. Table S25 presents the raw data used for the ecotoxicological test of prochloraz using *M. ocellatus* cells. **c** *M. ocellatus* cells were administered with abamectin at half the EC_{50} for 6 h in medium containing 1% FBS. Then, neutral comet assays were performed. For each condition, the experiment was repeated three times, resulting in three independent biological data points (biological replicates). Each of biological triplicates was derived from analyzing 23 different samples. Furthermore, each of biological triplicates was reported using mean and standard deviation. $^{**}p < 0.01$ for two-way ANOVA followed by Bonferroni post–hoc test. Table S26 presents the raw data used for the genotoxicity assessment of abamectin using *M. ocellatus* cells

C. carpio cells (130.712 ppm) was higher than that used in *M. ocellatus* cells (124.336 ppm). For 2,4,6-trichlorophenol, 387.616 ppm was used in *M. ocellatus* cells and 410.942 ppm in *C. carpio* cells. The comet tail length was longer in *C. carpio*, which may be because the 2,4,6-trichlorophenol concentration used in *C. carpio* cells (410.942 ppm) was higher than that in *M. ocellatus* cells (387.616 ppm). For roxithromycin, 351.531 ppm was used in *M. ocellatus* cells and 346.488 ppm was used in *C. carpio* cells. The comet tail length was longer in *M. ocellatus* cells, which may be because the roxithromycin concentration used in *M. ocellatus* cells (351.531 ppm) was higher than that used in *C. carpio* cells (346.488 ppm).

The ecotoxicity and genotoxicity tests developed using *M. ocellatus* cells require refinement before final use by regulatory agencies such as the U.S. Environmental Protection Agency and the European Environment Agency. First, toxicity data obtained from ecotoxicity assessments should reflect the concentrations of toxicants detected in environments. The discrepancy between EC_{50} values obtained from ecotoxicity tests and those obtained in the environment can diminish the effectiveness of assessments in predicting whether a toxicant poses a substantial risk to the ecosystem [44]. In this study, EC_{50} values obtained from ecotoxicity assessments are in the hundreds of ppm. To investigate the discrepancy, we selected butachlor from the toxicants used in this study and conducted a literature review of butachlor concentrations observed in the environment. Concentrations of butachlor observed in the environment, such as surface water, have been reported to range from tens to hundreds of ppm, with one study showing concentrations ranging from 4,740 ppm to 118,850 ppm, exceeding regulatory limits such as the European Food Safety Authority guideline of 500 ppm [45]. The EC_{50} values for butachlor were 261.423 ± 38.125 ppm and 248.672 ± 27.004 ppm in *C. carpio* and *M. ocellatus* cells, respectively. Therefore, the EC_{50} value for butachlor is considered to be within the range of concentrations observed in the environment. For other toxicants besides butachlor, it should be confirmed whether the EC_{50} values obtained from ecotoxicity tests using *M. ocellatus* cells are within the range observed in the environment. If the EC_{50} value is outside the range observed in

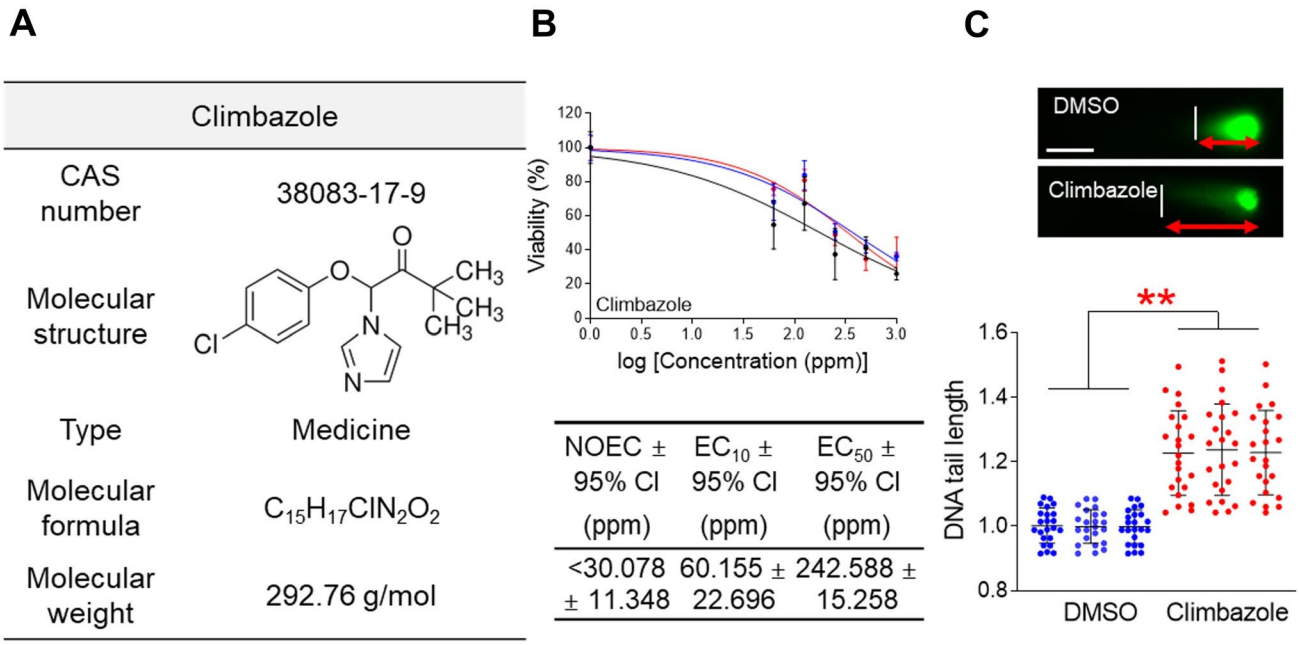


Fig. 11 Ecotoxicity and genotoxicity evaluation of climbazole using *M. ocellatus* cells. **a** The CAS number, molecular weight, type, molecular formula, and molecular structure of climbazole are presented. **b** *M. ocellatus* cells were administered with different concentrations of climbazole (0, 62.5, 125, 250, 500, and 1000 ppm) for 6 h in medium containing 1% FBS. Then, cell viability was assessed. For each condition, the experiment was repeated three times, resulting in three independent biological data points (biological triplicates). Each of biological triplicates was derived from analyzing 3 different samples. Furthermore, biological triplicates were reported using mean and 95% confidence interval (CI). The NOEC, EC_{10}, EC_{50}, and 95% CI were calculated using GraphPad Prism 9.0. Table S27 presents the raw data used for the ecotoxicological test of climbazole using *M. ocellatus* cells. **c** *M. ocellatus* cells were administered with climbazole at half the EC_{50} for 6 h in medium containing 1% FBS. Then, neutral comet assays were performed. For each condition, the experiment was repeated three times, resulting in three independent biological data points (biological replicates). Each of biological triplicates was derived from analyzing 23 different samples. Furthermore, each of biological triplicates was reported using mean and standard deviation. $^{**}p < 0.01$ for two-way ANOVA followed by Bonferroni post–hoc test. Table S28 presents the raw data used for the genotoxicity assessment of climbazole using *M. ocellatus* cells

the environment, the toxicant should be excluded from the analysis. Second, to obtain regulatory approval, standardized testing procedures must be used to accumulate in vitro data for ecotoxicity and genotoxicity assessment. These data should be used to scale in vitro results into indicators that can predict broader ecological impacts. This will assess the potential risks of toxicants to humans and the environment, enable preemptive management and regulatory decision-making, and ultimately contribute to regulatory approval.

In summary, this study developed a rapid ecotoxicity and genotoxicity assessment using *M. ocellatus* cells. This assessment method showed similar ecotoxicity and genotoxicity results to the existing assessment method using *C. carpio* cells. Moreover, this assessment platform has been demonstrated to be a universal assessment tool for a variety of substances by reproducing the genotoxicity of a variety of toxicants previously known to be genotoxic. Here, we propose that this novel platform provides a new paradigm for ecotoxicity and genotoxicity assessment and can be utilized as an effective tool to protect aquatic ecosystems from toxic substances.

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Data availability The original contributions presented in the study are included in the article; further inquiries can be directed to the corresponding authors.

Declarations

Conflict of interest The authors declare no conflicts of interest. The funders had no role in the collection, analyses, or interpretation of

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