Received: 12 March 2024 | Revised: 21 April 2024 | Accepted: 1 May 2024

DOI: 10.1002/appl.202400032

RESEARCH ARTICLE

Applied Research

Development of a label-free, impedance-based biosensor to identify harmful effects of pesticides on insect cells

Sandra Friedrich[1] | **Neha Malagimani**[1] | **Stefanie Michaelis**[2] | **Joachim Wegener**[1,2]

[1]Institute of Analytical Chemistry, Chemo- and Biosensors, University of Regensburg, Regensburg, Germany

[2]Fraunhofer EMFT, Fraunhofer Institute for Electronic Microsystems and Solid State Technologies, Regensburg, Germany

Correspondence
Joachim Wegener, Institute of Analytical Chemistry, Chemo- and Biosensors, University of Regensburg, Regensburg, Germany.
Email: Joachim.Wegener@ur.de

Abstract

Insects are a major part of the planet's ecosystem and their vital role as pollinators for agriculture is undisputed. Alongside factors as climate change or loss of habitats, rising use of pesticides emerges as a key threat to insect populations. For fighting this man-made problem, development of an easy, fast, sensitive, and non-invasive biosensor for determining pesticide toxicity may help to ban harmful substances and formulations. Here, a biosensor based on Sf21 (*Spodoptera frugiperda*) insect cells as sensors and electric cell-substrate impedance sensing (ECIS) as physical transducer is described. Sf21 cell suspensions and well-defined pesticide solutions were mixed immediately before seeding on planar gold-film electrodes. The capacitance at 20 kHz was recorded as a function of time as a measurand for cell adhesion providing dose–response profiles of pesticide impact. For future in-field applications, decoupling of the cell culture routines from the actual cytotoxicity assay is mandatory. Thus, suspensions of Sf21 cells were cryopreserved at −80°C in the wells of multielectrode arrays and thawed anytime for conducting the assays. Five pesticides were tested for their concentration-dependent cytotoxicity expressed as EC_{50} values by ECIS and validated using the well-established WST-1 cell viability assay. Results were found to be in good agreement. Our studies revealed cytotoxic effects of some pesticides sold for home usage far below the recommended concentration and were found to be more toxic than formulations sold for agricultural industry only.

KEYWORDS

biosensor, cryopreservation, cytotoxicity, ECIS, insect cells, pesticides, smart farming, whole-cell biosensing

INTRODUCTION

The awareness of humans for environmental protection increased in recent years, triggered by accumulating news about environmental catastrophes and climate change [1]. In particular declining insect population, especially bees, became widely known [2]. Research revealed that humans play a significant role in these threatening developments [3]. Habitat destruction, the climate change in general and the increasing application of environmental toxins such as pesticides cause insect populations to decline [4]. In 2017, the

Krefeld Society report unveiled a general decline in insect biomass by 75% in 26 years [5] with pesticides emerging as a key contributor to this trend [3, 4]. This enormous decline triggered the need for pollination by hand in some regions [6]. Pollination represents the most notable contribution of insects to the ecosystem in general and agriculture in particular. But there is more. For instance, insects control ecosystem cycles, improve soil quality by decomposition of organic waste acting as "recyclers" [7, 8] and they control other insect populations themselves serving as natural pesticides [9]. Bottom line, there are many obvious and truly important reasons to protect insects and their diversity for the planet's ecosystem [10]. Thus, research on cultured insect cells to test for cytotoxic effects of pesticides has attracted considerable interest.

So far, only invasive and label-based cytotoxicity assays with insect cells exposed to pesticide formulations or their active ingredients have been reported. Based on the label-based colorimetric MTT assay (3-(4,5-dimethylthiazol-2-yl)−2,5-diphenyltetrazolium bromide), the cytotoxic impact of various insecticides' active ingredients were studied with *Sf9/Sf21* cells, derived from *Spodoptera frugiperda* (fall armyworm) [11], *S2* cells, derived from *Drosophila melanogaster* (fruit fly) and *High Five* cells derived from *Trichoplusia ni* (cabbage looper) [12, 13]. The MTT assay is known to mirror metabolic activity of the cells under test [14]. Besides this metabolic MTT assay, several other different label-based assays have been applied, as for instance, the Comet assay to determine genotoxic effects of λ-cyhalothrin [15] or an enzyme-based assay to monitor cell membrane damage caused by bendiocarb and chlorpyrifos [16]. Those assays have in common that they are label-based and operate as end-point assays with no temporal resolution, neglecting the kinetic aspect of toxic reactions [14, 17]. To overcome potential assay limitations due to inherent biological activity of the labels themselves and the lack of temporal information, we applied label-free and time-resolved impedance measurements according to the ECIS principle (ECIS = *electric cell-substrate impedance sensing*, commercially available from Applied BioPhysics Inc.) to monitor the response of insect cells to pesticide exposure.

ECIS is based on growing adherent cells on small planar gold-film electrodes deposited on the bottom of culture dishes. The electrodes serve as growth surface for the cells and electrochemical transducer [18] at the same time. A weak alternating current (AC) in the µA range is applied at a distinct frequency in the range of 10 Hz–100 kHz [19] between a small working electrode (diameter 250 µm) and an approximately 500–1000-fold bigger counter electrode [20–22]. The cell culture medium serves as electrolyte closing the electric circuit [23]. Due to the dielectric properties of the plasma membrane, the cell bodies behave as insulating particles forcing the current to flow around (<10 kHz) or through (>10 kHz) the cell bodies depending on the AC frequency (see Figure 1) [23]. By selecting the proper AC frequency, ECIS enables assessing several highly relevant cellular phenotypes, like cell-cell or cell-substrate contact formation [24], cell migration, cell proliferation and cell death [25, 26] with high temporal resolution but without using any assay reagents [18, 19, 26].

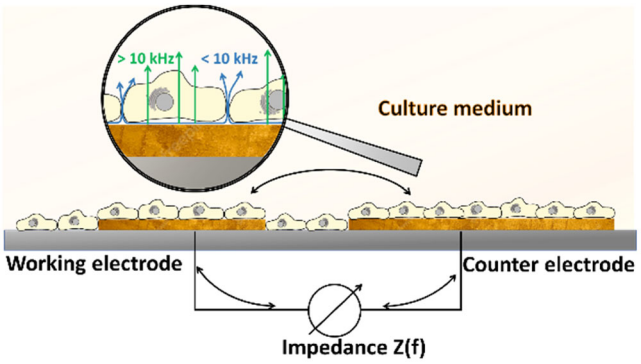

FIGURE 1 Basic principle of electric cell-substrate impedance sensing (ECIS).

Luong et al. [27, 28] were the first to combine insect cell cultures with an ECIS readout. Sf9 cells were seeded on gold-film electrodes to assess cytotoxicity of $HgCl_2$ and three explosives from ECIS-based resistance measurements [27, 28]. Later, Male et al. determined "EC_{50}" values for Sf9 cells exposed to steroids, [27] graphene derivatives [28], *Antrodia camphorata* isolates [29] and TiO_2 nanoparticles [30]. To the best of our knowledge, ECIS has never been used in combination with insect cells to study insect-specific response profiles to pesticides. The latter is in the focus of this study to help fostering the development of safer and environmentally friendly pest control strategies preventing the use of pesticides that are harming beneficial insects [31, 32].

In this study, we seeded Sf21 cells, originally derived from the ovary tissue of *Spodoptera frugiperda*, known as fall armyworm, on ECIS electrodes to quantify the cytotoxicity of various pesticides from their impact on cell adhesion kinetics. For future in-field applications, we explored decoupling of cell culture routines from the actual cytotoxicity assay by freezing suspensions of Sf21 cells at −80°C in the wells of multi-electrode arrays and thawing them immediately before pesticides were added for testing. Results recorded for fresh cells and formerly frozen cells are validated using the well-established, metabolic WST-assay (water-soluble tetrazolium salt).

METHODS

Cell line and culture conditions

Suspended Sf21 cells (provided by the Department of Biochemistry III of the University of Regensburg from the lab of Prof. Dr. Gernot Laengst) were cultured in glass Erlenmeyer flasks using serum-free medium (Sf-900TM II SFM; Thermo Fisher Scientific GmbH). Cell suspensions were routinely kept in a shaking incubator (100 rpm, 27°C) with a non-humidified atmosphere without CO_2 supplementation. In general, cells and all solutions applied to cells were handled between 26°C and 28°C, if not stated otherwise. Furthermore, cultivation and handling of cells was performed under a sterile bench

using sterile equipment and materials. Subcultivation was performed every 2 or 3 days by dilution with fresh culture medium into a new Erlenmeyer flask reaching a density of (0.2–0.6) × 10^6 cells/mL. Cells used for experiments were centrifuged for 10 min at 110*g* before aspirating the supernatant and re-suspending the remaining cell pellet in fresh culture medium. After counting, cells were diluted to the appropriate density using fresh culture medium.

Preparation of pesticide samples

Five commercially available pesticide formulations were studied: (i) insecticide Careo® (active ingredient acetamiprid 5 g/L; Schädlingsfrei Careo® Konzentrat; SUBSTRAL® Scotts Celaflor® GmbH), (ii) insecticide Bi 58®N (active ingredient λ-cyhalothrin 0.75 g/L; Bi 58® Insektenvernichter N; COMPO GmbH), (iii) fungicide Fonganil® Gold (active ingredient metalaxyl-M 465.15 g/L; Syngenta Agro GmbH), (iv) fungicide Saprol® (active ingredient triticonazole 9.72 g/L; CELAFLOR® Rosen-Pilzfrei Saprol®; Scotts Celaflor® GmbH) and (v) herbicide Banvel® M (active ingredients MCPA (2-methyl-4-chlorophenoxyacetic acid) 340 g/L and dicamba 30 g/L; Celamerck GmbH & Co. KG). For cytotoxicity studies, all formulations were diluted in cell culture medium and the pH was set to 6.34, the optimal pH for Sf21 cells, followed by a sterile filtration and preparation of a dilution series. In samples with a concentration termed 0% (v/v) pesticide, only cell culture medium was present. The osmolarity of solutions with the highest pesticide concentration was determined using a freezing point osmometer (Osmomat 030; Gonotec). The results were close to isotonic conditions (osmolarity of the culture medium is 0.379 Osmol/kg) and ranged between 0.340 Osmol/kg and 0.390 Osmol/kg. This range of osmolarities is easily tolerated by insect cells, [33] thus adjustment of osmolarity was not necessary.

WST-1 cell viability assay

The viability of Sf21 cells exposed to the various pesticide solutions was independently assessed using the WST-1 cell proliferation and viability assay (Roche Diagnostics Deutschland GmbH). The cells (3×10^5 cells/cm^2) and pesticide solutions in various concentrations were mixed in the wells of a 96-well plate before incubation. Absorbance was measured at 450 nm for 5 h every 30 min using the microplate reader Tecan Sunrise Basic. Wells containing culture medium only were used as blanks, whereas Sf21 cells exposed to 0.05% (v/v) Triton-X-100 in culture medium served as a *dead control*.

ECIS-based cytotoxicity assay

All ECIS experiments have been conducted using the ECIS 1600 R platform (Applied BioPhysics Inc.) and electrode arrays holding eight individual wells with 0.8 cm^2 growth area each. Each well contained a working electrode of 5×10^{-3} cm^2 surface area resulting from ten circular openings (d = 250 µm) in a photoresist passivation layer atop a continuous gold film (type 8w10e, Applied BioPhysics Inc.). Before the experiments, the electrode arrays were sterilized by argon plasma exposure for 30 s, followed by addition of 200 µL/well L-cysteine solution (10 mM in Millipore H_2O; containing 150 mM NaCl; pH 7.2; Sigma). After incubation for approximately 20 min, the wells were washed with Millipore water and culture medium. After washing, 400 µL/well of culture medium was added to each well and a cell-free baseline was recorded at a constant monitoring frequency of 20 kHz. After stabilization of the electrode impedance, suspensions of Sf21 cells were mixed with pesticide solutions in various concentrations in the wells of the electrode array to reach a final and constant cell density of 3×10^5 cells/cm^2. Immediately after cell seeding, the capacitance at 20 kHz was monitored for approximately 24 h. The ECIS device returns the the complex impedance Z. The complex impedance Z was subdivided in its real Re(Z) and imaginary component Im(Z). The imaginary component is interpreted as capacitive impedance that is then turned into an equivalent capacitance for the monitoring frequency of f = 20 kHz according to Equation (1).

$$C = \frac{1}{2\pi \times f \times Im(Z)}. \quad (1)$$

This data interpretation corresponds to treating the whole electrochemical system like a resistor and a capacitor in series. In the current manuscript, we used this equivalent capacitance to monitor cell attachment and spreading as we have detailed in previous papers [18, 22].

Cryopreservation of suspended Sf21 cells in wells of ECIS electrode arrays

Electrode arrays were prepared as described above. After washing with culture medium, they were cooled down to approximately 4°C. Suspensions of Sf21 cells were prepared in cooled culture medium (4°C) providing a final cell density of 6×10^5 cells/cm^2. Cell density was adjusted twice as high as compared to work with fresh cells to account for possible cell loss during cryopreservation. 5% (v/v) dimethyl sulfoxide was used as cryoprotective additive. The final cell suspension (100 µL/well) was added to the wells of the electrode array (Figure 2a), the electrode arrays were frozen to −80°C (Figure 2b) and stored at −80°C for various time periods from 7 to 56 days before the cells were used in ECIS-based cytotoxicity assays.

For performing an ECIS-based cytotoxicity assay the electrode arrays were thawed at 27°C for 5 min. Subsequently, the pesticide solutions were added to the wells (Figure 2c) and mixed with the cell suspension by gentle pipetting before the cells were allowed to settle. Capacitance was continuously recorded at a sampling frequency of 20 kHz for 24 h to monitor cell adhesion.

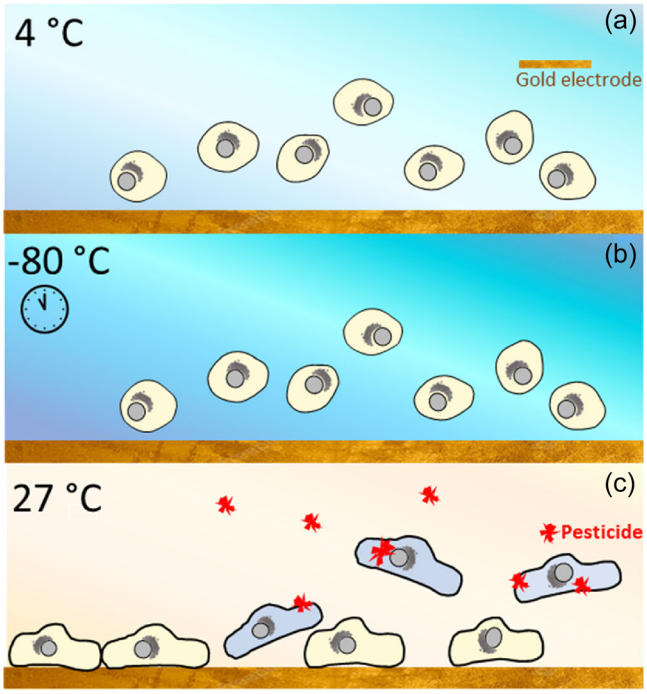

FIGURE 2 Schematic presentation of the cryopreservation and thawing process of Sf21 cell suspensions in wells of ECIS electrode arrays. (a) Cell seeding at 4°C. (b) Cryopreservation at −80°C. (c) Thawing and pesticide sample addition at 27°C.

RESULTS AND DISCUSSION

ECIS-based cytotoxicity assay for various pesticide formulations

The ECIS-based cytotoxicity assay presented here monitors differences in cell adhesion kinetics in presence and absence of harmful compounds. Figure 3 summarizes experimental raw data and subsequent data processing by example of the herbicide Banvel® M, which contains the active ingredients dicamba and 2-methyl-4-chlorophenoxyacetic acid (MCPA).

The capacitance time courses are normalized to the first capacitance reading after cell addition to the well (= time zero) and plotted with linear (Figure 3a) or logarithmic (Figure 3b) time scale, respectively, for the total assay time of 24 h. The logarithmic time scale is particularly useful to visualize the very prominent differences in cell adhesion kinetics early on within the first 3 h of the experiment.

We have shown before that the electrode capacitance above a threshold frequency of approximately 10 kHz, depending on electrode size and cell type, mirrors coverage of the electrode by adhering cells [18] and, thus, cell adhesion. Time course data in Figure 3a starts with the capacitance of the cell-free electrode and changes gradually to the capacitance of the cell-covered electrode. The latter is significantly lower than the former due to the presence of the cellular plasma membranes with their rather low dielectric constant. The slope of the curve is a measure for the adhesion rate, whereas the final capacitance values reflect the strength of cell adhesion to the electrode. Lower values of the normalized capacitance, after adhesion is complete, indicate tighter adhesion of the cells with smaller distances between cell membrane and electrode surface. When the first derivative of the capacitance time course is plotted as a function of time (Figure 3c), the minimum of the curve provides the maximum rate of adhesion. It proved to be a direct but relative measure for Sf21 cell viability. The faster the capacitance decreases and the more pronounced the minimum of the first derivative is, the faster the cells attach on the electrodes. For Banvel® M concentrations up to 0.006% (v/v), the attachment rate of Sf21 cells is not significantly affected relative to an untreated control (0% (v/v)). A significant impact of Banvel® M on attachment rate and strength of adhesion is observed for concentrations between 0.03% (v/v) and 0.06% (v/v). For concentrations higher than 0.06% (v/v), cells do not attach anymore indicating that these concentrations are critically harmful and may even cause cell death. To describe the harmful impact of the pesticide more quantitatively, we extracted a dose–response relationship (Figure 3d, red curve) from the *area-under-the-curve* (AUC) between the individual capacitance time courses and a baseline of integration at a normalized capacitance of 1.2 for the first 5 h of the experiment. AUC values for the different concentrations of Banvel® M were normalized to the AUC value recorded for the control cells. Fitting a four-parameter logistic function (Equation 2) to the dose–response data provided an EC_{50} value of (0.041 ± 0.0018) %(v/v) for Banvel® M (Figure 3d).

An identical experiment was performed with Sf21 cells that had been cryopreserved in the wells of the electrode array for 7 days before they were thawed, mixed with pesticide solutions, and analyzed with respect to their adhesion kinetics. Data recording and processing as described in the previous section provided an EC_{50} value of (0.035 ± 0.0017) % (v/v) which is only slightly lower than the EC_{50} value observed for cells that were not frozen right before the assay (Figure 3d, blue curve). According to these experiments, the outcome of the assay is independent of the cryopreservation of the cells. Thawing them, immediately before the assay is conducted, does not have any impact on the sensitivity of the cells for the pesticide Banvel® M. Table 1 compares EC_{50} values recorded with "fresh" or "frozen" cells using four other pesticides in the ECIS-based viability assay.

WST-1 cell viability assay

To compare the ECIS-based analysis with a standard viability assay, we exposed Sf21 cells to increasing concentrations of Banvel® M and all reagents of the well-established WST-1 assay. WST-1 reads the metabolic activity of the cells by a colorimetric reaction monitored by absorbance readings. Figure 4a displays the time courses of absorbance when Sf21 cells were exposed to different concentrations of the herbicide Banvel® M. As observed in ECIS experiments, cell viability decreases with increasing concentration of Banvel® M, as the slopes of

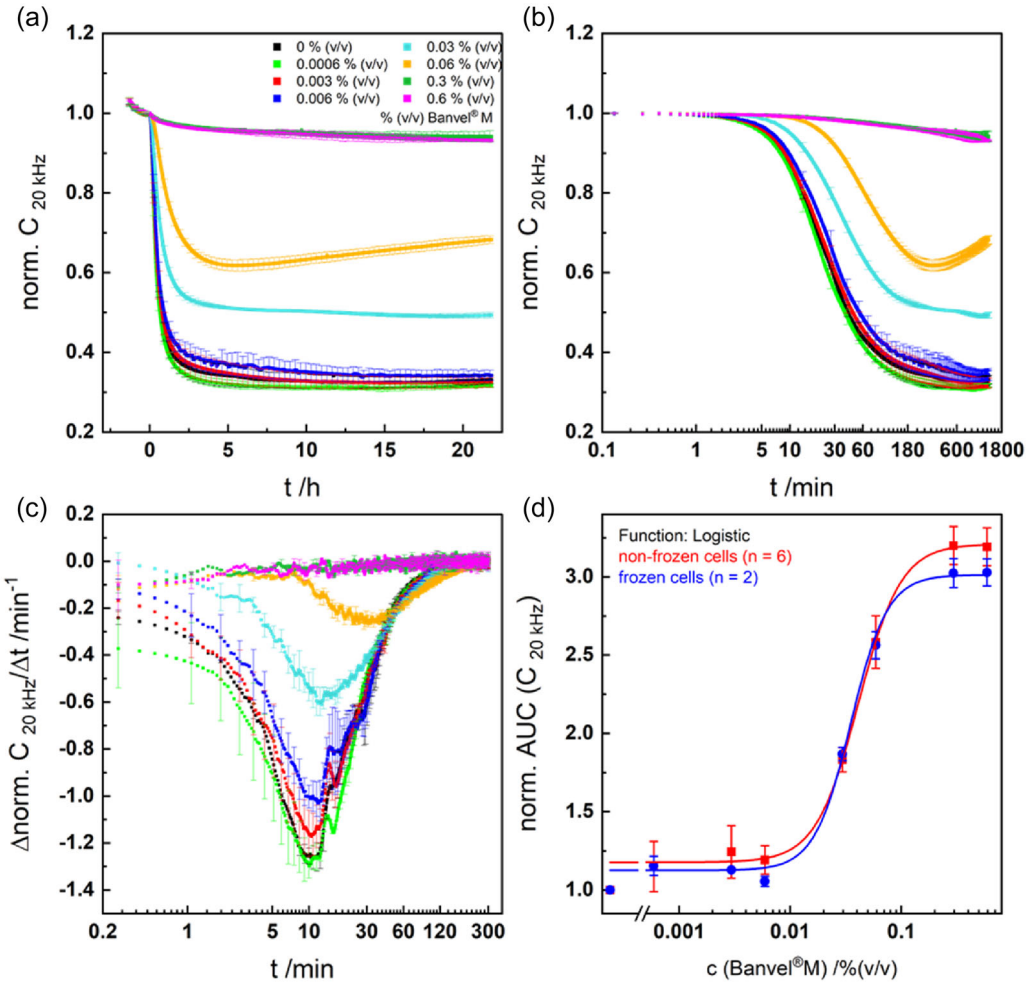

FIGURE 3 Time-resolved normalized capacitance at 20 kHz for different Banvel® M concentrations in cell culture medium with linear (a) and logarithmic (b) timescale. Sf21 cells were seeded with a density of 3×10^5 cells/cm^2 without cryopreservation ($n = 2$). (c) First derivative of A plotted with a logarithmic timescale, ($n = 2$). (d) Area under the curve (AUC) values, obtained by integration of the time courses, normalized to the untreated control and fitted with a logistic function with instrumental weighting of the errors. In red: non--frozen Sf21 cells seeded with a density of 3×10^5 cells/cm^2, $R^2 = 0.99941$, ($n = 6$). In blue: Cells cryopreserved on the electrode arrays for seven days before thawing and addition of the Banvel® M, $R^2 = 0.99433$, ($n = 2$). Data was fitted with the logistic fit function (Equation 2). Red curve: lower asymptote A1 = 1.18 ± 0.02; upper asymptote: A2 = 3.21 ± 0.02; power: p = 2.3 ± 0.15; center: EC_{50} = (0.041 ± 0.001) % (v/v); blue curve: A1 = 1.125 ± 0.005; A2 = 3.01 ± 0.09; $p = 2.6 \pm 0.7$; EC_{50} = (0.035 ± 0.0025) % (v/v). All data are given as mean ± SEM.

the absorbance time courses decrease for increasing concentration. Higher absorbance values reflect higher cell viability, as the WST-1 chromogen is only reduced to a chromophore by cellular oxidoreductases when cell metabolism provides sufficient reducing power (NADH, NADPH) [17]. The dose–response curve (Figure 4b) was established by integration (AUC) between the individual time courses and an integration baseline of A_{450}-A_{620} = 1.5 from time zero to the end of the experiment after 5 h. Individual AUC values were normalized to AUC for the untreated control. Similar to the ECIS experiment, a four-parameter logistic function (see Equation (2)) was fitted to the dose–response data and the fit returned an EC_{50} value of (0.035 ± 0.0015) % (v/v) for Banvel® M ($n = 3$). In the logistic function, A_1 and A_2 denote the upper and lower asymptote of the fit, respectively. EC_{50} is the x-value of the point of inflection of this curve corresponding to the concentration of half maximum efficacy. The parameter p denotes the Hill slope reporting on cooperative effects in the dose–response relationship.

$$y = \frac{A_1 - A_2}{1 + \left(\frac{c}{EC50}\right)^p} + A_2. \quad (2)$$

The result compares favorably with the EC_{50} value recorded by ECIS-based analysis of cell adhesion.

Testing different classes of pesticides by means of the ECIS-based cytotoxicity assay

The same experiments as described for Banvel® M were conducted with four other pesticides for "fresh" cells and those that were cryopreserved before. Two different storage times at –80°C were studied: (i) short-term storage for 7–12 days or (ii) more long-term storage between 26 and 56 days. The raw data recorded from both, ECIS- and WST-1-based viability assays,

TABLE 1 Summary of EC_{50} values for five pesticide formulations given as mean ± SEM in % (v/v) determined with the WST-1 cell viability assay, ECIS-based cytotoxicity assay with non-nfrozen cells, and cells that were cryopreserved before the assay for time periods given in brackets.

Pesticide formulation	Active ingredient	WST-1 EC_{50} ± SEM/% (v/v) (n = 3)	ECIS non-frozen cells EC_{50} ± SEM/% (v/v) (n = 3–6)	ECIS frozen cells EC_{50} ± SEM/% (v/v) (freezing duration in days) (n = 2)	Concentration of use as recommended by manufacturer/% (v/v)
Banvel® M	MCPA + dicamba	0.035 ± 0.0015	0.041 ± 0.0018	0.035 ± 0.0017 (8) 0.031 ± 0.0018 (37)	0.06–3 [34]
Careo®	Acetamiprid	0.98 ± 0.05	1.0 ± 0.12	1.2 ± 0.18 (7) 0.7 ± 0.016 (56)	1.00–6.00 [35]
Bi 58®N	λ-Cyhalothrin	0.55 ± 0.011	0.396 ± 0.007	0.60 ± 0.024 (7) 0.52 ± 0.04 (44)	2.00 [36]
Fonganil® Gold	Metalaxyl-M	0.027 ± 0.0014	0.0216 ± 0.0009	0.029 ± 0.0018 (12) 0.034 ± 0.003 (50)	0.013 [37]
Saprol®	Triticonazole	(1.7 ± 0.13) 10^{-6}	(21 ± 0.26) 10^{-6}	(14 ± 1.6) 10^{-6} (7) (8 ± 2.3) 10^{-6} (26)	1.00 [38]

Note: The values are compared to the pesticide concentration recommended by the manufacturer for use in the field.
Abbreviations: ECIS, electric cell-substrate impedance sensing; MCPA, 2-methyl-4-chlorophenoxyacetic acid; SEM, standard error of mean.

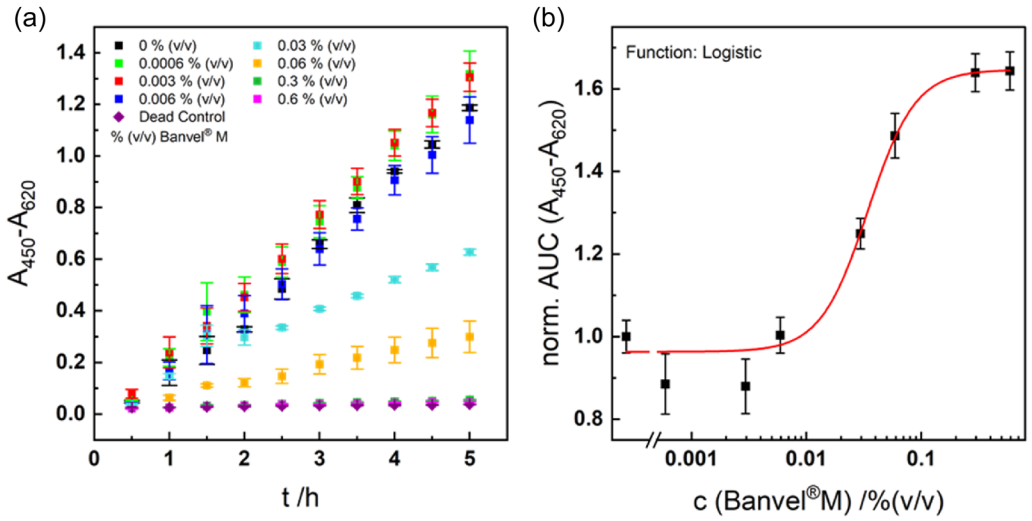

FIGURE 4 (a) Time resolved A_{450}-A_{620} absorbance values of the WST-1 viability assay, corrected by the absorbance of a blank only containing cell culture medium. The absorbance values were determined every 30 min over a total measurement period of 5 h. The assay was done with Sf21 cells in suspension that were seeded with a density of 3×10^5 cells/cm^2 per well and exposed to different Banvel® M concentrations in cell culture medium. The positive (*dead*) control contains Triton-X-100 (0.05% (v/v) in culture medium). (b) AUC values, obtained by integration of the time courses, normalized to the untreated control and fitted with the logistic function (Equation 2) with instrumental weighting of the errors, R^2 = 0.98604, (n = 2). Parameters of logistic fit: A_1 = 0.96 ± 0.028; A_2 = 1.65 ± 0.04; p = 2.1 ± 0.7; EC_{50} = (0.034 ± 0.004) % (v/v). All data are given as mean ± SEM. AUC, area under the curve; SEM, standard error of mean.

were processed and analyzed as described above. EC_{50} values are compared in Table 1.

For Careo®, an insecticide with acetamiprid as the presumably active ingredient, EC_{50} values of all assays are in good agreement independent of the assay (ECIS vs. WST-1) or the history of the cells. Just the EC_{50} value returned by the ECIS-assay for cells stored at −80°C for 56 days is slightly lower, indicating higher sensitivity of the cells. The experimental EC_{50} values correspond to the lower end of the recommended concentration range of use. Thus, this formulation shows the half maximal harmful impact on Sf21 cells if used in the lowest concentration recommended by the manufacturer. For comparison, a literature study [11] reports an IC_{50} value of 0.2329 mM acetamiprid from MTT assays using Sf9 cells as reporters. Sf9 cells are a clonal isolate from Sf21 cells [39]; MTT is a colorimetric assay based on the same principle as WST-1 [11]. Assuming that the cytotoxic effect of Careo® is due to acetamiprid as the active ingredient, it is useful to calculate the concentration of acetamiprid from the EC_{50} value of Careo® given in % (v/v). As the commercial formulation contains 5 g/L acetamiprid [35], an

EC_{50} of 1.0 % (v/v), as returned by the assays conducted in this study, corresponds to approx. 0.22 mM acetamiprid in good agreement with the literature data [11].

For Bi 58®N, an insecticide containing the (presumably) active ingredient λ-cyhalothrin, the EC_{50} value (all in % v/v) increases slightly from cells that were not frozen before the assay (0.396 ± 0.007) compared to those frozen for 7 days (0.60 ± 0.024) or 44 days (0.52 ± 0.04), respectively. However, considering the result of the WST-1 assay (0.55 ± 0.011) and the fact that working with cells on various days leads to inevitable differences, the results are close enough to underline the usefulness and reliability of an ECIS-based pesticide assay. Experimental EC_{50} values were found to be a factor of four lower than the minimum concentration recommended for use by the manufacturer. If cell culture experiments with Sf21 cells are reasonably predictive for whole insects, the recommended concentration of use is highly toxic for the latter. Assuming the cytotoxic effect is based on the active ingredient λ-cyhalothrin only, it is reasonable to transform the EC_{50} values in µM of λ-cyhalothrin. The values range between 6.6 µM for ECIS with non-frozen cells and 10 µM for the cells frozen for seven days. Compared to an EC_{50} value of (38.4 ± 4.3) µM for Sf9 cells exposed to λ-cyhalothrin as determined with the MTT assay [40], the EC_{50} values determined here are lower by a factor of approx. three to six, indicating a slightly lower sensitivity of Sf9 cells than Sf21 cells.

With respect to the ECIS assay, similar results were obtained for the fungicide Fonganil® Gold, containing metalaxyl-M as the (presumably) active ingredient. Just the cells cryopreserved for 50 days before the assay returned a slightly higher EC_{50} value compared to the use of "fresh" cells. Experimental EC_{50} values extracted from the assays of this study were found to be a factor of approximately two higher than the recommended concentration for use [36, 37] Thus, if applied in the recommended concentration and assuming cell culture assays to be predictive for whole insects, the use of Fonganil® Gold has only a moderate impact on insects.

The fungicide Saprol® based on the (presumably) active ingredient triticonazole showed by far the lowest EC_{50} value expressed in % (v/v) going below the ppm limit. Both assays, ECIS and WST-1, report similarly low values even though they differ by a factor of ten. We can only speculate on the origin of this difference in toxic potency within the two assays performed here. But it seems straightforward to assume that triticonazole has a direct inhibitory effect on oxidoreductase activity as indicated by others just recently [41]. Saprol® was the only pesticide in this study for which the two types of assays reported EC_{50} values that were more than 20% different. The differences between "fresh" cells and those cryopreserved for 7 or 26 days are insignificant. Much more relevant is the striking difference between experimental EC_{50} values and the recommended concentration of use. Sf21 cells were harmed by the pesticide at concentrations that were lower by a factor of 10^5 to 10^6 than the recommended concentration. This indicates an extremely toxic formulation for insects even if only cells rather than intact insects were studied here. Assuming that living insects are affected only 1000-fold less than Sf21 cells due to protection by, for example, their chitin shell [42], the formulation would still be tremendously harmful to the insects if used in the recommended concentration. For the pesticide formulations Fonganil® Gold, Saprol® and Banvel® M as well as for the pure active ingredients no literature data of toxicity studies with insect cells are known so far.

CONCLUSION

Results of the established WST-1 cell viability assay confirm data obtained from the ECIS-based viability assay reporting on changes in cell adhesion kinetics. EC_{50} values from both assays compared favorably even though both assays report on very different cell phenotypes. However, the ECIS assay is completely automated, does not require any labels that might affect cell physiology itself and it provides a time resolution that allows for in-depth analysis of the cell response. Upscaling the assay format from 8-well to 96-well format is straightforward without losing any of the technical advantages mentioned above. 96-well formats will enable even high throughput screening campaigns for new pesticides' active ingredients or new pesticide formulations and promote their chemical improvement. So far, the concept has only been applied to Sf21 cells. To increase information depth, the assay will be adapted to other insect species in the future as, for example, bee cells. This is of utmost importance since the loss of bee populations is a well-known threat to the environment and mankind. Since it is at least partially caused by pesticide use in agriculture, the assay described here may provide a first line of defense by early identification of bee-unfriendly formulations. Combinations of cell lines from different insect species on one electrode array to be used in one assay for a given pesticide provide an even more informative and unique toxicity statement. The concept of freezing suspended sensor cells directly in the wells of the electrode array and thawing them just minutes before conducting the assay provides reliable data that are very much in line with the use of "fresh" cells that were not frozen before the assay. This enables a time and cost-efficient preparation of batches of sensor cells weeks or even months before the time point when the assay is conducted. Thus, performing the assay becomes entirely independent of synchronized cell culture work that inevitably requires a laboratory environment and may pave the way for in-field applications of the assay technology described here.

ACKNOWLEDGMENTS

The authors acknowledge financial support by the German Federal Ministry of Education and Research (BMBF) and Bavarian Ministry of Economic Affairs, Regional Development and Energy (StMWi) in the initiative "Biogene Wertschöpfung und Smart Farming" conducted by the *Fraunhofer Society*. The project was

also supported by the High Performance Center Secure Intelligent Systems (LZSiS). We are very much indebted to Prof. Gernot Laengst who provided the Sf21 cells used in all assays. Open Access funding enabled and organized by Projekt DEAL.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Joachim Wegener http://orcid.org/0000-0001-8554-0150

REFERENCES

[1] United Nations Department for Economic and Social Affairs, *Sustainable Development Goals Report 2020*: United Nations, **2020**.
[2] M. A. P. Lima, G. C. Cutler, G. Mazzeo, M. Hrncir, *Front. Ecol. Evol.* **2022**, *10*, 1027169.
[3] J. P. van der Sluijs, *Curr. Opin. Environ. Sustain.* **2020**, *46*, 39.
[4] S. A. Dar, M. Javed Ansari, Y. Al Naggar, S. Hassan, S. Nighat, S. Burjes Zehra, R. Rashid, M. Hassan, B. Hussain, *Global Decline of Insects*, InTech Open, London **2022**.
[5] C. A. Hallmann, M. Sorg, E. Jongejans, H. Siepel, N. Hofland, H. Schwan, W. Stenmans, A. Müller, H. Sumser, T. Hörren, D. Goulson, H. de Kroon, *PLoS One* **2017**, *12*, e0185809.
[6] A. Wurz, I. Grass, T. Tscharntke, *Basic Appl. Ecol.* **2021**, *56*, 299.
[7] A. Jankielsohn, *Adv. Entomol.* **2018**, *06*, 62.
[8] G. Scudder, *Insect Biodiversity*, Blackwell Publishing Ltd, Hoboken **2017**, pp. 9–43.
[9] D. Goulson, *Insect Declines and Why They Matter*, The Wildlife Trust, Somerset.
[10] E. Wilson, *The Diversity of Life*, W.W. Norton, New York **1992**.
[11] M. Saleh, A. Rahmo, J. Hajjar, *Lebanese Sci. J.* **2013**, *14*, 115.
[12] X. Yun, Q. Huang, W. Rao, C. Xiao, T. Zhang, Z. Mao, Z. Wan, *Ecotoxicol. Environ. Saf.* **2017**, *137*, 179.
[13] N. Pandya, B. Thakkar, P. Pandya, P. Parikh, *J. Basic Appl. Zool.* **2021**, *82*, 60.
[14] A. Adan, Y. Kiraz, Y. Baran, *Curr. Pharm. Biotechnol.* **2016**, *17*, 1213.
[15] M. Saleh, J. Hajjar, A. Rahmo, *Jordan J. Biol. Sci.* **2021**, *14*, 213.
[16] J. Polláková, J. Pistl, N. Kovalkovičová, T. Csank, A. Kočišová, J. Legáth, *Pol. J. Environ. Stud.* **2012**, *21*, 1001.
[17] R. Parboosing, G. Mzobe, L. Chonco, I. Moodley, *Med. Chem.* **2016**, *13*, 13.
[18] J. Wegener, C. R. Keese, I. Giaever, *Exp. Cell Res.* **2000**, *259*, 158.
[19] S. Lukic, J. Wegener, *Encyclopedia of Life Sciences*, John Wiley & Sons, Hoboken **2015**, pp. 1–8.
[20] C. R. Keese, J. Wegener, S. R. Walker, I. Giaever, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 1554.
[21] J. A. Stolwijk, K. Matrougui, C. W. Renken, M. Trebak, *Pflügers Archiv. - Eur. J. Physiol.* **2015**, *467*, 2193.
[22] A. Janshoff, A. Kunze, S. Michaelis, V. Heitmann, B. Reiss, J. Wegener, *J. Adhes. Sci. Technol.* **2010**, *24*, 2079.
[23] B. Freiesleben De Blasio, J. Wegener, *Encyclopedia of Medical Technologies*, John Wiley & Sons, Inc, Hoboken.
[24] L. Robilliard, D. Kho, R. Johnson, A. Anchan, S. O'Carroll, E. Graham, *Biosensors* **2018**, *8*, 56.
[25] T. Iwakura, J. A. Marschner, Z. B. Zhao, M. K. Świderska, H.-J. Anders, *Nephrol. Dial. Transplant.* **2021**, *36*, 216.
[26] M. Zinkl, J. Wegener, *Curr. Opin. Environ. Sci. Health* **2019**, *10*, 30.
[27] K. B. Male, S. M. Crowley, S. G. Collins, Y.-M. Tzeng, J. H. T. Luong, *Anal. Methods* **2010**, *2*, 870.
[28] K. B. Male, E. Lam, J. Montes, J. H. T. Luong, *ACS Appl. Mater. Interfaces* **2012**, *4*, 3643.
[29] K. B. Male, Y. K. Rao, Y.-M. Tzeng, J. Montes, A. Kamen, J. H. T. Luong, *Chem. Res. Toxicol.* **2008**, *21*, 2127.
[30] K. B. Male, M. Hamzeh, J. Montes, A. C. W. Leung, J. H. T. Luong, *Anal. Chim. Acta* **2013**, *777*, 78.
[31] G. Smagghe, C. L. Goodman, D. Stanley, *In Vitro Cell. Dev. Biol. - Animal* **2009**, *45*, 93.
[32] X. He, L. Lu, P. Huang, B. Yu, L. Peng, L. Zou, Y. Ren, *Insects* **2023**, *14*, 104.
[33] E. Schlaeger, *Cytotechnology* **1996**, *20*, 57.
[34] Land24 GmbH, *Pflanzenschutzmittel: Banvel M*. https://www.raiffeisen.com/pflanzenschutzmittel/detail/050023-00 (accessed: January 18, 2024).
[35] Land24 GmbH, *Pflanzenschutzmittel: Schädlingsfrei Careo Konzentrat*. https://www.raiffeisen.com/pflanzenschutzmittel/detail/005686-00 (accessed: January 18, 2024).
[36] Land24 GmbH, *Pflanzenschutzmittel: Bi 58 N*. https://www.raiffeisen.com/pflanzenschutzmittel/detail/007028-60 (accessed: January 18, 2024).
[37] Syngenta, *Product information Fonganil® Gold*. https://www.syngenta.de/sites/g/files/kgtney356/files/media/document/2023/07/05/Gebranleitung_Fonganil%20Gold_Art%2053-Zierpflanzen_MB-SD_23Mai2023_0.pdf (accessed: January 18, 2024).
[38] Land24 GmbH, *Pflanzenschutzmittel: Rosen-Pilzfrei Saprol*. https://www.raiffeisen.com/pflanzenschutzmittel/detail/004582-00 (accessed: 18.01.24).
[39] I. Cameron, *Trends Biotechnol.* **1989**, *7*, 66.
[40] B. R. Ahissou, W. M. Sawadogo, A. H. Bokonon-Ganta, I. Somda, M.-P. Kestemont, F. J. Verheggen, *Afr. Entomol.* **2021**, *29*, 435–444.
[41] D. L. Roman, D. I. Voiculescu, M. A. Matica, V. Baerle, M. N. Filimon, V. Ostafe, A. Isvoran, *Molecules* **2022**, *27*, 6554.
[42] H. Merzendorfer, L. Zimoch, *J. Exp. Biol.* **2003**, *206*, 4393.

How to cite this article: S. Friedrich, N. Malagimani, S. Michaelis, J. Wegener, *Appl. Res.* **2024**, e202400032. https://doi.org/10.1002/appl.202400032