In Vitro Technics for Determination of the Eye Irritant Properies of the Agrochemicals

In vitro technika az agrokemikáliák szemirritációs tulajdonságának meghatározására

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Abstract: Until now the most popular method to classify substances' eye irritation potential is the OECD 405 test guideline. The basis of this is the Draize-test, which is one of the most criticized in vivo methods, because of the injuries of the test animals and subjective nature of the test in recording the results. Nowadays, several alternative tests are available which can be partly or totally replaced the in vivo eye irritation testing depending on the circumstances. The Isolated Chicken Eye Test (ICET) is part of these alternative methods. Four different agrochemicals (Biscaya, Dezormon, Kyleo, Pulsar 40 SL) were examined with this in vitro method. In ICET the eye irritation potential of test items were predicted based on the combination of three endpoints: corneal swelling, corneal opacity and fluorescein retention. The basis of determination of each endpoint was the differences between values of the base line measurement and values of any observation time points after the post-treatment rinse. Three agrochemicals showed different severity irritation potential and one agrochemical did not show any effect on the treated corneas. Comparing these in vitro results with the available in vivo data of the tested agrochemicals, results are found to be corresponding to each other.

Keywords*: eye irritation; in vitro; corneal swelling; corneal opacity; fluorescein retention*

Összefoglalás: A Draize-féle primer szemirritációs tesztet nagyon sok kritika éri az eredmények szubjektív értékelése, de legfőképp a vizsgálatok során felhasznált állatok szenvedése miatt. A napjainkban elérhető alternatív módszerek a körülményektől függően részben vagy akár teljes mértékben képesek kiváltatni az in vivo tesztet. Ezen eljárások közé tartozik az izolált csirkeszem vizsgálatán alapuló szemirritációs vizsgálati módszer (ICET). Ezen vizsgálati módszer elvégzése során a kezelést követően a szaruhártya-duzzadás, -homály és fluoreszein megtartás mértékét az alap értékhez viszonyítva határoztuk meg, és az így kapott végpontok kombinációiból következtettünk a vizsgálat agrokemikáliák (Biscaya, Dezormon, Kyleo, Pulsar 40 SL) szemirritációs potenciáljára. A vizsgált agrokemikáliák közül három eltérő mértékben, de szemirritáló tulajdonságúnak mutatkozott, míg egy vizsgálat anyag esetében nem állapítottunk meg szemirritációs potenciált az alkalmazott in vitro módszer alapján. A rendelkezésre álló in vivo eredményekkel összehasonlítva azt tapasztaltuk, hogy az összes vizsgált anyag esetében a saját in vitro és az in vivo eredmények megegyeztek.

***Kulcsszavak**: szemirritáció; in vitro; szaruhártya-homály; szaruhártya-duzzadás; fluoreszein megtartás*

1. Introduction

In the Isolated Chicken Eye Test (ICET) the agrochemicals are applied in a single dose onto the cornea of isolated chicken eyes. The purpose of this test is to classify the agrochemical as ocular corrosive and/or severe irritant (UN GHS Category 1) or to identify the test item as a chemical that does not require classification for eye irritation (UN GHS No Category) or serious eye damage under the UN GHS classification system.

The ICET does not fully replace the in vivo Draize-test (OECD 405), because it cannot be identified of the agrochemicals, that should be classified as slight or moderate eye irritant (UN GHS No Category 2). The reason of lack of this ability, that the necessary physiological properties of the chicken eyes can be hold on only few hours after the chickens slauthered, but day or weeks are necessary for the examination of the reversibility of the observed effects (Buda et al., 2013). However, this method is used as part of a tiered testing strategy for regulatory purposes (Budai et al., 2004; Tavaszi and Budai, 2006; Tavaszi et al., 2008).

Four different agrochemicals (Biscaya, Dezormon, Kyleo, Pulsar 40 SL) were tested with this method. Our goal was to determinate how the results, which got this in vitro test method, can be collerate with the available in vivo test results and based on that how effectively use this in vitro test system for the determination of the eye irritation properties of the agrochemicals in the future.

2. Materials and Methods

The Isolated Chicken Eye Tests were performed based on the OECD 438 (2023) guideline.

Chicken heads collection and transport: Breed of chicken was ROSS 308. The heads were transported at the earliest convenience for use approximately within 2 hours from collection.

Selection and preparation of eyes for the test: Corneal integrity was checked by fluorescein solution. Eyes that had high baseline fluorescein staining or corneal opacity score or any additional signs of damage after enucleation was rejected. A minimum of seven eyes were used for each test (three treated eyes, three positive control eyes and one negative control eye).

The base line assessments: Baseline values were required to evaluate any potential agrochemicals related effects after treatment.

Treatment: Biscaya (Bayer Hungária Ltd., Hungary), Dezormon (Nufarm Hungária Ltd., Hungary), Kyleo (Nufarm Hungária Ltd., Hungary) and Pulsar 40 SL (BASF Hungária Ltd., Hungary) were applied onto the centre of the cornea (standard amount was 30µL) such that the entire surface of the cornea was covered.

Test item removal: After an exposure period of 10 seconds from the end of the application, the cornea surface was rinsed thoroughly with ~20 mL isotonic saline at ambient temperature.

Observation: The cornea thickness and opacity of all eyes (control and test eyes) were evaluated pre-treatment and at approximately 30, 75, 120, 180 and 240 minutes after the post-treatment rinse. The fluorescein retention was measured on two occasions, baseline (t=0) and 30 minutes after the post-treatment rinse.

Evaluation: The endpoints evaluated were corneal opacity, swelling, fluorescein retention, and morphological effects (e.g., pitting or loosening of the epithelium).

3. Results

The purpose of ICETs was to evaluate the potential ocular corrosivity and irritancy of the agrochemicals Biscaya, Dezormon, Kyleo and Pulsar 40 SL by their ability to induce toxicity in enucleated chicken eyes:

The overall ICE classes of the Pulsar 40 SL treated corneas were thrice I (based on corneal swelling of 3 % within 240 minutes, based on the corneal opacity score of 0.5 and based on the fluorescein retention of 0.3) in the experiment.

The overall ICE classes of the Biscaya treated corneas were thrice II (based on corneal swelling of 8 % within 240 minutes, based on the corneal opacity score of 1.3 and based on the fluorescein retention of 1.3) in the experiment.

The overall ICE classes of the Kyleo treated corneas were thrice III (based on corneal swelling of 19 % within 240 minutes, based on the corneal opacity score of 1.7 and based on the fluorescein retention of 2.0) in the experiment.

The overall ICE classes of the Dezormon treated corneas were thrice IV (based on corneal swelling of 48 % within 240 minutes, based on the corneal opacity score of 3.7 and based on the fluorescein retention of 3.0) in the experiment.

The positive control was classified as corrosive/severely irritating, UN GHS Classification: Category 1 in each experiment. The negative control had no significant effects on the chicken eye in these tests and was categorized as UN GHS Non-Classified in each experiment. So, the positive and negative controls showed the expected results in each experiment and confirmed the validity, sensibility and suitability of the tests.

4. Discussion

The Pulsar 40 SL did not show eye irritation property in this in vitro eye irritation test, which result is harmonizing with the available in vivo result (see Table 1).

The agrochemicals Biscaya and Kyleo have been categorized as ‘no prediction can be made’. This means, that they did not cause serious eye damage, but they exact eye irritation properties cannot be determined with this method, because the lack of the observation of the reversibility processes prevents the identification of the agrochemicals, which should be classified as slight or moderate eye irritant (UN GHS No Category 2). However, these results are perfectly harmonized with the available in vivo results (see Table 1).

The Dezormon showed serious eye damage. This means it can be classified as Category 1, which is the same as the available in vivo result (see Table 1).

Table 1. The in vitro irritation categories of the tested agrochemicals obtained in in vitro tests and the in vivo eye irritation categories on the safety data sheets

Agrochemicals	In vitro GHS[1] Classification based on the ICET results	In vivo GHS[1] Classification based on the SDS[2]
Pulsar 40 SL	No Category	No Category
Biscaya	No prediction can be made	Category 2
Kyleo	No prediction can be made	Category 2
Dezormon	Category 1	Category 1

[1]*GHS: Globally Harmonized System*

[2]*SDS: Safety Data Sheet*

According to the results above, the available in vivo date are fully supported by the in vitro isolated chicken eyes tests.

Based on the correlation between the in vivo and in vitro results, it can be established in accordance with the opinion of other authors (Adriaens et al., 2014; Budai et al., 2021), that The Isolated Chicken Eye Test method is applicable for, that partly or totally replace the in vivo test method with its weakness of the lack of the observation of reversibility processes.

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