TESTING OF PESTICIDES FOR EYE IRRITATION WITH THE HET-CAM TEST

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Abstract

There is an ongoing effort to replace the *in vivo* Draize test, which was generally and exclusively accepted for decades - taking into account the 3R rule and animal welfare regulations - with different alternative *in vitro* methods. One of these *in vitro* methods is the test using the chorioallantoic membrane of the hen's egg (HET-CAM). The plant protection agents included in our studies were determined using this method, and then the results were compared with the *in vivo* data. During the HET-CAM test, the plant protection agents were applied directly to the chorioallantoic membrane and the changes in the blood vessel system were observed. From the obtained results, it can be concluded that the results of the HET-CAM test closely approximate the *in vivo* data.

Keywords: eye irritation, pesticide, chorioallantoic membrane, HET-CAM test

Összefoglaló

Az évtizedekig általánosan és kizárólagosan elfogadott *in vivo* Draize-teszt kiváltására - a 3R szabály és az állatvédelmi előírások figyelembevételével - a törekvés folyamatos különböző

alternatív *in vitro* módszerekkel. Az egyik ilyen *in vitro* módszer a tyúktojás chorioallantois membránját felhasználó teszt (HET-CAM). Vizsgálatainkba bevont növényvédő szereket ezzel a módszerrel határoztuk meg, majd az eredményeket összevetettük az *in vivo* adatokkal. A HET-CAM teszt során a növényvédő szereket közvetlenül juttattuk a chorioallantois membránra és figyeltük meg a vérérrendszer elváltozásait. A kapott eredményekből megállapítható, hogy a HET-CAM teszt eredményei jól közelítik az *in vivo* adatokat.

Kulcsszavak: szemirritáció, növényvédő szer, choriollantois membrán, HET-CAM teszt

Introduction

Toxicological eye irritation tests are an important part of the licensing process for plant protection products. With the help of these, it is possible to know exactly the harmful effects on the eyes. For decades since 1944, Draize's primary eye irritation test was the only method to accurately determine these harmful effects. However, this procedure can be painful for the experimental animal and is ethically highly questioned by animal protection organizations, so in accordance with the 3R rule based on (Russell and Burch 1959), alternative *in vitro* methods aimed at induction have appeared one after another. Today, several such methods based on isolated eyes or tissue cultures are accepted by the OECD, but none of them can cover the entire irritation potential, namely cannot reliably indicate all three GHS categories. GHS categories are: helath hazards, physical hazards and environmental hazards. Health hazards are threats to human health (e.g. breathing or vision), while physical hazards harm the body (e.g. skin corrosion) and environmental hazards include pollution and natural disasters like hurricanes and earthquakes that have the potential to endanger the environment or have a negative impact on human health. During our study, hatched hen eggs were carefully opened on the 10th day so that the chorioallantoic membrane (CAM) could be observed. The chorioallantoic membrane is

a suitable tissue for evaluating eye irritation properties because it reacts similarly to the rabbit eye and can be technically easily studied (Leighton et al., 1985).

We compared the results from the observed changes caused by plant protection agents with the *in vivo* data, with the aim of which was to see how close the two methods are to each other, and to expand the data in this direction.

Material and method

The *in vivo* data were taken from the safety data sheet of the products.

The four plant protection products Tilmor (fungicide), Prosaro (fungicide), Zantara (fungicide), Kideka (herbicide) used were applied in 100% concentration in HET-CAM test.

The HET-CAM test was performed based on Invittox Protocol No. 47. The breeding eggs were obtained from the premises of Gallus Kft. Hatching took place at 37.5 ˚C and 50-70% relative humidity in a Ragus type incubator. The eggs were turned several times a day (Spielmann, 1997) to prevent the embryo from sticking. During the preparation, the calcareous shell of the hen's eggs that became manageable on the 10th day was cut around and removed above the air chamber with surgical forceps. The membrane was moistened with an avian physiological NaCl solution, then carefully pulled up with surgical forceps, thus making the chorioallantoic membrane suitable for the treatment. As a negative control, 2 eggs were treated with avian physiological NaCl solution, and as a positive control, 2 eggs were also treated with a 1% sodium lauryl sulfate (SDS) and 0.1 M NaOH solution. For each test material, the treatment was carried out with 4 repetitions on 6 eggs. 0.3 ml of test substance was dropped onto the membrane and observed for 5 minutes after treatment. As a result of the treatments, the starting time of the changes appearing on the membrane (bleeding, blood vessel lysis or coagulation) was recorded to the nearest second. Using the algorithm included in the protocol, irritation

indices were determined based on the type and onset time of the recorded changes, and then the test substances were classified into *in vitro* irritation classes with the help of these.

Results

During the treatment with Tilmor fungicide, lysis was observed on the membrane between 17-40 seconds, which was not followed by bleeding in all cases. Based on the obtained irritation index, the pesticide is irritative.

After applying the Prosaro fungicide to the membrane, lysis occurred from the 15th second, which was not followed by bleeding. The product was classified as irritating.

After treatment with Zantara fungicide, lysis was observed between 16-27 seconds, followed by bleeding between 80-140 seconds. Based on the results obtained, the product is irritating.

During the application of Kideka herbicide, lysis was recorded from the 19th second and bleeding from the 180th second. The product was classified as irritating.

Discussion

Table 1 shows the comparison of the results of the HET-CAM test with the *in vivo* data.

Table 1 Comparison of the categories of the HET-CAM test and in vivo data

Test materials	HET-CAM test irritation category	*in vivo* data
Tilmor	irritant	irritant
Prosaro	irritant	irritant
Zantara	irritant	irritant
Kideka	irritant	severely irritant

Based on the results of the four pesticides included in our study, we can conclude that the data are well correlated. There is a 75% match. We reached a similar result as Bagley et al. (1992; 1994), Kormos (2011) and Jírová et al. (2014), who established during their research that the

HET-CAM test provides the lowest rate of false results and also provides valuable results related to the conjunctiva.

The HET-CAM test has advantageous properties compared to *in vitro* methods: it has adequate sensitivity, is cheaper, and is faster. Disadvantages include the subjectivity of evaluation and the influence of the physical and chemical properties of the test materials on the evaluation. During our further work, we therefore included other alternative *in vitro* methods in our research.

Acknowledgement

We would like to thank Bayer and Nufarm for providing us with the plant protection products.

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