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THE USE OF THE RADIOISOTOPE METHOD IN STUDIES OF PESTICIDE PENETRATION INTO THE EYEBALL

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Abstract: The studies concerning the effects of pesticides on the human body focused mainly on their local action or chronic poisoning of the organism. In this study we examine the pesticide penetration into the eyeball resulting from direct contact with the eye. We used an isotope-labelled carbamate pesticide - carbaryl. The determinations of the amount and concentration of this substance in the cornea, aqueous humour, vitreous humour and retina were performed using the method measuring β radiation emitted by radioactive carbon. The results revealed measurable concentrations of labelled carbamate in the cornea and aqueous humour 10 and 30 min after application. The levels of this pesticide in the vitreous humour and retina were very low and difficult to analyse statistically. The described method appears to be useful in determining the range and rate of eye penetration by environmental toxins having direct contact with the eyeball.

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INTRODUCTION

The synthetic pesticides introduced as crop protection products in the 1940s significantly affected an increase in agricultural production. However, it took 20 years of their common use to notice the side effects of their action on the environment and human organisms [3]. The latest data from WHO indicate that one million accidental pesticide poisonings occur each year world-wide [6].

Many groups of pesticides are distinguished, including: insecticides, herbicides, fungicides and rodenticides. The active ingredients of these products are mainly organophosphates, carbamates, chlorinated hydrocarbons, carbamide derivatives [6, 9].

Poisonings with pesticides are likely to occur through inhalation, food, skin or mucous membranes. The disorders may result from long-term exposure to toxins [1] or direct contact of tissues with these compounds [5]. Moreover, it should be remembered that pesticides may affect the visual system [2, 4]. However, the papers concerning this issue available in ophthalmologic literature are rare; only single cases of optic neuritis [10], optic injuries [8] and accomodative asthenopia [7] resulting from long-lasting exposure to pesticides have been described. Direct contact of eye tissues with pesticides is likely to cause eyeball irritation, conjunctiva or/and cornea burns [1, 5].

Since pesticides are in common use, many people, especially farmers are exposed to accidental eyeball injuries caused by these compounds. Therefore, the aim of the study was to determine whether pesticides, in addition to causing the above-mentioned local changes, may penetrate the eyeball.

MATERIALS AND METHODS

The compound studied was [^{14}C]-carbaryl ([^{14}C]-1-naphthyl-N-methylcarbamate; 99.8%; 400 MBq/g) obtained from the Centre of Isotope Production and Distribution in Świerk (Poland). The pesticide was administered to each eyeball in the volume of 0.15 ml. Two concentrations of carbaryl were used: 40% and 2%. The 40% solution is a concentrate available on the market while the 2% solution

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is used for spraying. In both concentrations, the radioactivity was adjusted with cold carbaryl to 75 kBq/ 0.15 ml. The recovery was determined using the standard radioactive solution of [^{14}C]-n-hexadent (51.4 kBq/g) purchased from the Institute of Nuclear Studies in Świerk (Poland).

The experiments were performed in 9 rabbits of both sexes weighing 2–2.5 kg fed with a standard laboratory mixture. Food and water were available *ad libitum*. The rabbits were divided into 2 groups. The first consisted of 4 rabbits (8 eyeballs) who received 2% suspension of labelled carbaryl. In 2 rabbits of this group (4 eyeballs) the exposure time was 10 minutes, in the other two (4 eyeballs) – 30 minutes. The second group also consisted of 4 animals (8 eyeballs); the procedure was the same but the concentration of carbaryl was 40%. One rabbit (2 eyeballs) served as control. The conjunctival sac was anaesthetised with 0.25% pantocaine and 0.15 ml of carbaryl was administered to both eyes of all 8 rabbits. After the exposure time (10 and 30 minutes), the conjunctival sac was washed thoroughly with 0.9% NaCl to remove the pesticide. The rabbits were subjected to euthanasia according to the standard procedure and the samples of the anterior chamber humour, cornea and vitreous humour with retina were collected. The tissue was placed in the balanced scintillating vessel and weighed with the accuracy of 0.1 mg; then 1ml of the macerating solution (NSC- II Tissue Solubilizer, Amersham) was added and the samples left for 24 h at room temperature. After that period the samples were placed in the shaker with water bath of 350 shakes/min at 50ºC for 2 hours. Then they were cooled and 5 ml of scintillator added. The material was left in a dark place for 48 h and its radioactive activity measured using a β counter (LSC, Wallec model 1409). The measurement time for each sample was 5 minutes. The yield of the counter, of the samples used for background determinations and of those with the radioactive standard, was determined by performing 20 measurements in each case. The number of measurements for the eye tissue samples exposed to carbaryl was 5. The results were presented as the number of counts per minute per 100 mg tissue (CPM/100 mg).

The eye tissue samples collected from the rabbit not exposed to carbaryl were used to determine the background levels. A series of measurements of samples containing the eye tissue with the added radioactive solution (n-hexadecan), scintillator and standard was performed to determine the quantitative impulse suppression caused by the tissues in the scintillating fluid. The detection values observed in the anterior chamber humour samples decreased by 18.28% while those in the samples containing the cornea and vitreous humour + retina were found to be decreased by 18.80% and 17.68%, respectively. The results were expressed as mean ± standard deviation and statistically analysed using the Student t-test at significance level of $p < 0.05$.

RESULTS

The rabbits exposed to local action of 2% carbaryl for 10 minutes showed high penetration of this compound into the anterior chamber – 5869 CPM/100 mg tissue. After 30 minutes of exposure, this level significantly decreased to 509 CPM/100 mg tissue. Similarly, application of 40% suspension of carbaryl resulted in radioactivity level of 4431 CPM/100 mg tissue and 1157 CPM/100 mg tissue after 10 and 30 min of exposure, respectively. After administration of 2% carbaryl the radioactivity detected in cornea reached 12273 CPM/100 mg tissue after 10 min and 3111 CPM/100 mg tissue after 30 min of exposure. Application of 40% carbaryl produced corneal radioactivity level of 54360 CPM/100 mg tissue and 15064 CPM/100 mg tissue after exposure lasting 10 and 30 min, respectively.

The values of carbaryl found in the vitreous humour, retina and vascular membranes were low and difficult to analyse statistically and thus were omitted.

CONCLUSIONS

The results of our study lead to the following conclusions:

1. The measurements of recovery show low percentage of radiation suppression and indicate that the conditions of determinations were properly prepared.
2. The background values calculated for the anterior chamber humour and the vitreous humour with retina were similarly small while these values for the cornea were 10-fold higher.
3. In accordance with expectations, the highest accumulation level of the radioactive compound was found in the cornea; the lowest one - in the anterior chamber humour.
4. The vitreous humour and retina penetration was small and almost unrelated to the concentration of locally applied carbaryl.
5. In all the eye structures examined, especially in the aqueous humour, the radiation values were significantly lower after 30 minutes compared to those after 10 minutes, which indicates rapid pesticide elimination from the eyeball.
6. The described method appears to be useful in determining the range and rate of eye penetration by environmental toxins having direct contact with the eyeball.

REFERENCES

1. Balluz LS, Philen RM, Brock J, Falter K, Kiefer M, Hart R, Hill RH: Health complaints related to pesticide stored at a public health clinic. *Environ Res* 2000, **82**,1-6.
2. Dementi B: Ocular effects of organophosphates: a historical perspective of Saku. *J Appl Toxicol* 1994, **14**, 119-129.
3. Van der Werf HMG: Assessing the impact of pesticides on the environment. *Agriculture, Ecosystems and Environment* 1996, **60**, 81-96.
4. Ishikawa S: Ophthalmopathy due to environmental toxic substances especially intoxication by organophosphorus pesticides. *Nippon Ganka Gakkai Zasshi* 1996, **100**, 417-432.

5. Meulenbelt J, de Vries I: Acute work-related poisoning by pesticides in the Netherlands; a one year follow-up study. *Przegl Lek* 1997, **54**, 665-670.

6. O'Malley M: Clinical evaluation of pesticide exposure and poisonings. *Lancet* 1997, **349**, 1161-1166.

7. Piasecki M, Buratowski J, Warczyński A: Syndrome of ophthalmological manifestations caused by a generalized intoxication with defoliants and herbicides. *Pol Tyg Lek* 1974, **29**, 1567 (in Polish).

8. Stępień T: 2 cases of lesions of the organ of vision caused by chemicals some branches of industry and agriculture. *Klin Oczna* 1973, **43**, 169-172 (in Polish).

9. Trusiewiczowa D: Pesticides and the visual system. *Klin Oczna* 1983, **85**, 263-265 (in Polish).

10. Wasilewski M: The action of poisons in human environment and retrobalbular neuritis. *Klin Oczna* 1977, **47**, 229-231 (in Polish).