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Resistance of two Planarian Species to UV-irradiation

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The aim of this work was to determine the effects of 20, 25 and 30 minute UV-irradiation periods λ=253.5 nm to two planarian species *Dugesia tigrina* (Gir.) and *Polycelis felina* (Daly.). *In vivo*, UV light effects have been reported to affect intracellular receptors and disrupt simple behaviour. The effects of UV-rays on mortality and behavior as well as morphological, cytological and histological changes in the two planarian species were assessed, and the course and the dynamics of regenerative processes were compared between them. Experimental populations of *Dugesia tigrina* and *Polycelis felina* species were maintained in laboratory conditions at room temperature. Mortality, behavioral and morphological changes were monitored daily by means of a light stereomicroscope. For cytological and histopathological analysis, planarians were fixed in Bouine fixative on the first, second, third, fifth and seventh day after exposure to UV-irradiation, respectively. They were embedded in paraffin, cut on a microtome, stained with toluidin blue and embedded in Canada-balsam. UV-rays caused mortality, behavioral, morphological, cytological and histological changes in each planarian species. In regeneration of damaged body parts reticular cells and neoblasts played the main role. Neoblasts as totipotent cells extremely increased in number in the area of damaged tissue, immediately after UV-exposure. *Dugesia tigrina* was more sensitive to UV-rays than *Polycelis felina* due to possession of less pigmented cells. The course of regeneration in both species was similar. Most individuals of both species regenerated in 5 to 12 days after UV-irradiation.

Key words: Planarian, UV-irradiation, deformation, damage, repair, regeneration.

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Planarians are useful test organisms for laboratory research and may be maintained easily. In nature they are widely distributed and easily accessible. They abundantly inhabit clean mountain streams and shallow lake areas. In a short time interval, many experimental animals can be obtained. Extensive regeneration is exhibited by freshwater planarians, capable of regenerating a complete organism from any tiny body fragment, and consequently have attracted the interest of scientists throughout history. Equally impressive is the developmental plasticity of these Platyhelminthes, including continuous growth and fission (asexual reproduction) in well-fed organisms, and shrinkage (degrowth) during prolonged starvation. The source of their morphological plasticity and regenerative capability is a stable population of totipotent stem cells - neoblasts; this is the only cell type in the adult that has mitotic activity and differentiates into all cell types. This cellular feature is unique to planarians in the Bilateria clade (SALO 2006). Throughout their lifetime they remain in possession of neoblasts, undifferentiated, mitotically active totipotent cells that are capable of differentiation into any other cellular types that may be used or damaged. Reticular cells play a major role during regeneration of the damaged body parts. They appear in the wound area a few hours after the damage and also have the capability of phagocytosis (VILLAR & SCHAEFFER 1993). After the activation of reticular cells, neoblasts migrate into the woundarea.

Different agents such as heavy metals and herbicides given in sublethal doses cause behavioral, morphological, cytological and histological changes, as well as alterations in the mitotic cycle in planarians (FRANJEVIĆ *et al.* 2000; KALAFATIĆ & TABORŠAK; 1998, KALAFATIĆ & TOMAŠKOVIĆ; 1999, 2000; KOPJAR *et al.* 1997; MILIĆ-ŠATSUMURA & KALAFATIĆ 1997). It was also established by other aquatic test organisms that UV-irradiation of λ=253.5 nm wavelengths causes changes immediately after the irradiation, which are mostly regenerated in the short period of time. Ultraviolet (UV) light is electromagnetic radiation

with a wavelength shorter than that of visible light, but longer that soft X-rays. It can be subdivided into near UV (380-200 nm wavelength), far or vacuum UV (200-10 nm; abbrev. FUV or VUV) and extreme UV (1-31 nm; abbrev. EUV or XUV) (MATSUMURA & ANANTHASWAMY 2004). In combination with other agents UV-irradiation increases mortality, biomagnifies and causes more extreme cellular and tissue damages in planarians (KOPJAR *et al.* 1994). *In vivo*, UV light effects have been reported to disrupt simple behaviors such as spontaneous locomotor activity (RAFFA *et al.* 2003). Some recent studies suggest complex interactions of UV light with intracellular receptors in organisms, e.g. the dopamine D2-receptor antagonist sulpiride decreases spontaneous locomotor velocity of planarians (pLMV) in an enantiomeric-selective and dose-dependent manner and is significantly attenuated by UV light (254 and 366 nm) (RAFFA & MARTLEY 2005).

The mutagenic effects of UV-irradiation to DNA in *Drosophila melanogaster* and mitochondrial DNA in humans have been established (PASCUCCI *et al.* 1997; VAN DER HELM *et al.* 1997). In humans, prolonged exposure to solar UV radiation may result in acute and chronic health effects on the skin, eye, and immune system. Ultraviolet photons harm the DNA molecules of living organisms in different ways. In one common damage event, adjacent bases bond with each other, instead of across the "ladder". This makes a bulge, and the distorted DNA molecule does not function properly (GRANT 2002).

The aim of the present study was to determine the effects of UV-rays λ=253.5 nm on mortality, behavior as well as morphological, cytological and histological changes observed in the two planarian species in laboratory conditions and to compare the course and the dynamics of regenerative processes between them.

Material and Methods

Planarians of the *Polycelis felina* (Daly.) species were collected at the creek Gračanski ribnjak near Zagreb, Croatia and planarians of the *Dugesia tigrina* (Gir.) were obtained from the Laboratory for Ecotoxicology of the Faculty of Natural Sciences in St. Petersburg. Populations were maintained for years in laboratory conditions as a culture. Planarians were maintained in the dark, in aquarium water in Petri dishes (11 cm in diameter) at a temperature of 7 ^{o}C. They were fed once a week with the pieces of rat liver. One week before the experiment and during the experiment animals were not fed. Healthy animals of the same developmental stage and 10-12 mm in size were chosen for the experiment. A comparative toxicity test was used, where each experimental group consisted of 10 animals. One group of 10 animals of each planarian species served as a control group, while other groups of each planarian species were irradiated with UV-light λ=253.5 nm for exposure periods of 20, 25 and 30 minutes, respectively. Aquarium water of each experimental group was changed daily. Altogether the experiment with the recovery period for each planarian species lasted for 20 days and it was repeated 3 times. Each time similar results were obtained.

Mortality, behavioral and morphological changes were monitored daily by means of a stereomicroscope. The most damaged animals from each group were chosen for histological and cytological analysis. One animal per period (20, 25, 30 min) was fixed in Bouin fixative on the 1st, 2nd, 3rd, 5th and 7th day after irradiation, respectively and embedded into paraffin. Parrafined tissue was cut by microtome on 7 μm thick slides. Preparations were stained with 0.1 % toluidin blue and immediately embedded into Canada-balsam. Preparations were examined under a Reichert light microscope. Micrographs were taken by a Pentax camera, with Kodak color film.

Results

Mortality, locomotor and morphological changes in *Dugesia tigrina* irradiated with UV-rays λ=253.5 nm for periods of 20, 25 and 30 minutes

On the first day after exposure to UV-rays all the treated planarians were contracted and rested at the bottom of the glass dish. They reacted to the mechanical stimuli with water flush from a pipette by body movements around their body axis. Planarians in the control group showed tendency for more directed movement. In planarians irradiated for 20 min with UV-light of 253.5 nm wavelength, an accumulation of pigment was observed. Planarians irradiated for 25 min manifested more severe damage in the shape of wounds on the anterior part of the body. Planarians irradiated for 30 min were wounded all over the body and they had their epidermis folded on the lateral part of the body.

On the second day after the exposure to UV-rays, deformations in treated animals were more intense. All the planarians were contracted and rested at the bottom of the glass dish. They reacted shortly to the mechanical stimuli. Lateral parts of the body were still folded. Accumulation of pigment was detected on the anterior part of the body almost in all the treated planarians. Damage to

auricles was evident. In planarians irradiated for 20 min with UV-light of 253.5 nm wavelength accumulation of pigment was still observed. In almost all planarians irradiated for 25 and 30 minutes with UV-rays wounds were present all over the body. Damaged cells fell out of the planarian body and mucous leaked out.

On the third day after UV exposure in all experimental periods, manifestations of the described changes were still present, but with the less intensity. The treated planarians were mostly contracted, but a period of relaxations was noticed. Movements of irradiated animals were slower as compared to the control group. The distribution of the pigment was irregular. Depigmented areas were present as well as areas with increased accumulation of pigment.

On the fourth day after exposure to UV-rays, animals responded to the mechanical stimuli by a quicker reaction and for a longer period of time. Pigmentation in the planarians irradiated for 20 min was still irregular. The mortality rate was 10 % in planarians treated for 25 min. Wounds were noticeable on the posterior part of the body of planarians treated for 30 min.

On the fifth day after exposure to UV-rays in all experimental periods, regeneration was in progress. Wounds were closed. These areas were somewhat paler than the rest of the body. The treated planarians were moving, but were still slower than the control.

Between the 6th and 12th day after exposure to UV-rays in all experimental periods, regeneration was continuing and most of the treated and regenerated planarians did not differ from the control. Planarians with more severe damage mostly moved slower than the control.

Between the 12th and 15th day after the exposure to UV-rays in all experimental periods regeneration was completed. Morphology and behavior of the treated animals did not differ from the control.

Mortality, locomotor and morphological changes in *Polycelis felina* irradiated with UV-rays λ=253.5 nm for periods of 20, 25 and 30 minutes

On the first day after irradiation all the planarians exposed to UV-rays for 20 min morphologically were comparable to the control. Ten percent of planarians irradiated with UV-rays for 25 min had wounds on their posterior part, from which mucous leaked out of the body. The epidermis was wrinkled on the lateral part of the organism. Thirty percent of planarians irradiated with UV-rays for 30 min had wounds on the ventral part along the entire body. The epidermis was also wrinkled on the lateral part of the body. All the treated planarians had relaxed bodies. They moved only after mechanical stimuli with water flush from a pipette. Changes in pigmentation could be observed in most of the animals irradiated with UV-rays for 30 min.

On the second day after irradiation with UV-rays, damage was visible in 20 % of planarians irradiated with UV-rays for 20 min and 80 % of planarians irradiated with UV-rays for 30 min. Damage was mostly localized on the posterior ventral side of the body. Pigmentation of these animals was not regularly homogenous. The epidermis was wrinkled on the lateral parts of the body.

On the fourth day after irradiation wounds were completely closed. Most of the planarians started to move normally all over the bottom of the dish. Only 20 % of the planarians irradiated with UV-rays for 25 and 30 min moved slower than individuals and in the shorter intervals and the control.

Between the 5th and 12th day after irradiation regeneration was complete. The irradiated planarians did not differ from the control neither by morphological appearance nor by behavior.

Cytological and histological changes in *Dugesia tigrina* irradiated with UV-rays λ=253.5 nm for periods of 20, 25 and 30 minutes

On the first day after irradiation, the planarian body was contracted. The intestinal lumen was extremely reduced and partly filled with mucous, absent in the control. The outer mucous layer was damaged along the entire planarian body (Fig.1). No other changes in planarians irradiated with UV-rays for 20 min were observed. The epidermis was partly damaged in planarians irradiated with UV-rays for 25 and 30 min. Rhabdites were irregularly distributed and partly damaged. In wound areas mucous and damaged cells as well as an increased number of reticular cells were observed.

On the second day after irradiation, planarians were also contracted so the intestinal lumen was still reduced. Rhabdites were irregularly distributed in epidermis and many were damaged. In the wound area an increased number of neoblasts could be seen.

On the third day after irradiation in all the experimental periods the number of neoblasts was still increasing.

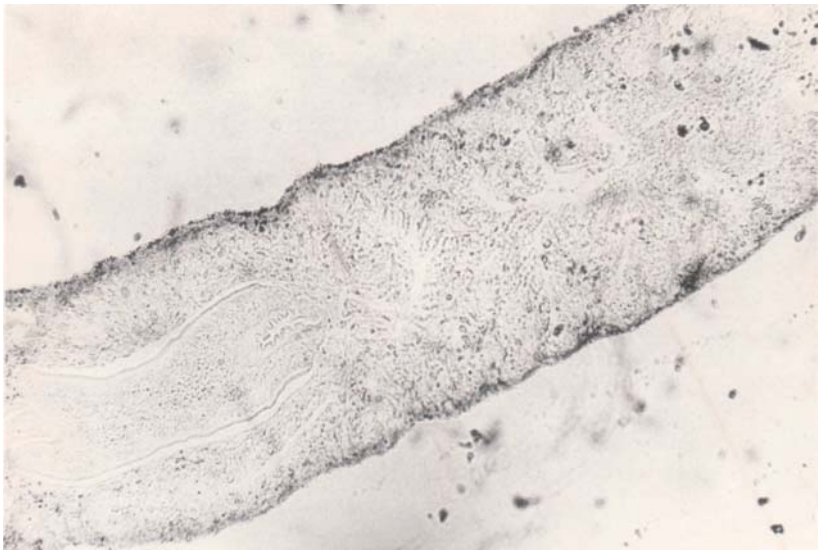

Fig. 1. Planarian *Dugesia tigrina* (Gir.) irradiated for 20 min with UV-rays of λ=253.5 nm and observed on the first day of the recovery period. Damage to the outer mucose layer and epidermis. Toluidin blue.

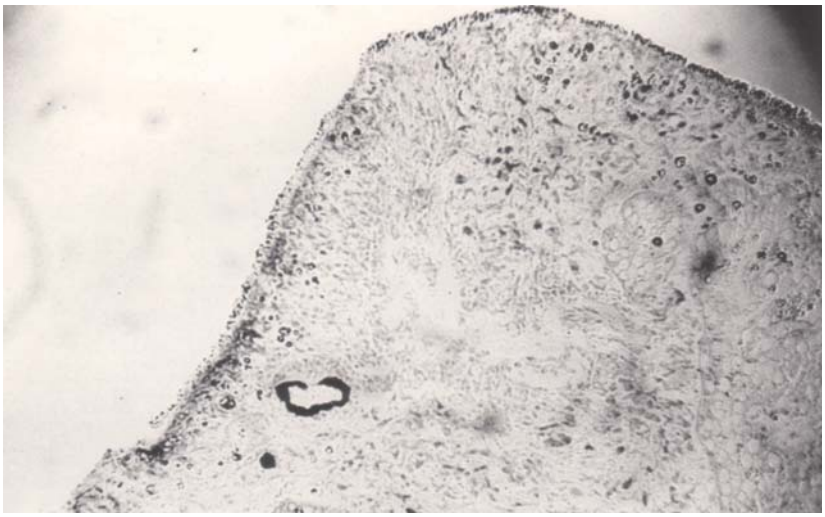

Fig. 2. Planarian *Dugesia tigrina* (Gir.) irradiated for 20 min with UV-rays of λ=253.5 nm and observed on the fifth day of the recovery period. Cyto-histological structure of planarian similar to control. Numerous neoblasts are present in parenchyma (arrow). Toluidin blue.

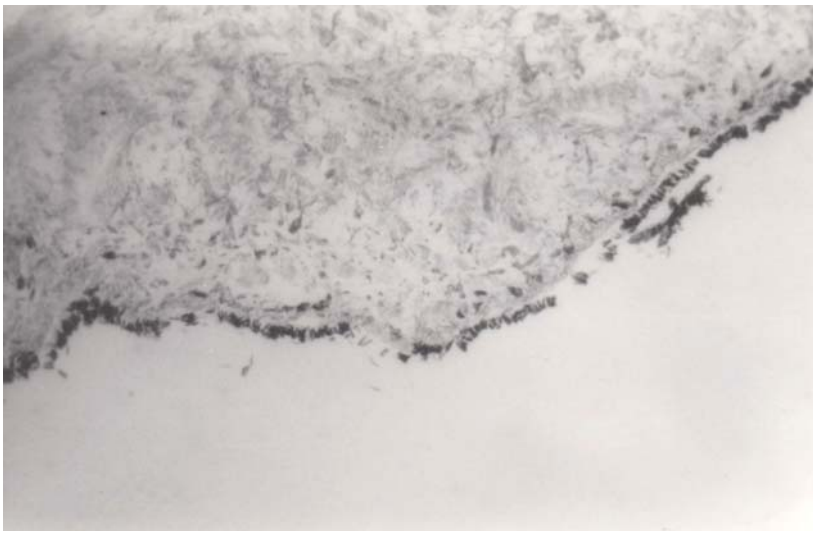

Fig. 3. *Polycelis felina* (Daly.) irradiated for 20 min and observed on the first day of the recovery period. Damage to epidermis is on the major part of the animal. Toluidin blue.

On the fifth day after irradiation planarians were relaxed and had a normal body shape. The intestines were similar to the control. The epidermis had for the most part a normal structure. On regenerated areas only epithelial cells without rhabdites were present. Numerous neoblasts were still present in the damaged area (Fig. 2).

On the seventh day after irradiation, cytological and histological composition of treated planarians did not differ from the control.

Cytological and histological changes in *Polycelis felina* irradiated with UV-rays λ=253.5 nm for periods of 20, 25 and 30 minutes

Only planarians treated for 25 and 30 min showed similar cytological and histological changes compared to the control.

On the first day after irradiation, damage to the mucous layer as well as epidermis could be seen (Fig. 3). Reticular cells were present in the wound areas and the epidermis was damaged to a large extent.

On the second day after irradiation, reticular cells were present. In the wound area numerous neoblasts could be found as well as much mucous.

On the third day after irradiation the number of neoblasts in damaged areas had increased.

On the fifth day after irradiation, damage was mostly regenerated. In the epidermis on some spots epithelial cells and occasionally rhabdites could be seen.

On the seventh day after irradiation, the cytological and histological structure did not differ from the control.

Discussion

Individuals from a *Dugesia tigrina* (Gir.) population irradiated with UV-light λ=253.5 nm for 20, 25, and 30 minute periods showed locomotor changes, as well as morphological, cytological and histological changes. Individuals from a *Polycelis felina* (Daly.) population irradiated with UV-light λ=253.5 nm for 20 minute period of time did not show any changes compared to the control. Locomotor, morphological, cytological and histological changes in *Polycelis felina* treated for 25 and 30 minute periods were less intense than in *Dugesia tigrina* treated for the same period, therefore they show a higher resistance UV-irradiation due to abundant pigmentation.

Behavioral changes were visible during the first three days after UV-ray treatment of planarians and were especially emphasized in *Dugesia tigrina*. Planarians were contracted and mostly remained still at the bottom of the glass dish. Upon mechanical stimuli with water flush they moved only for a very short period. *Polycelis felina* did not show this kind of locomotory inhibition induced by the UV-treatment. Lateral parts of the epidermis were folded on the posterior part of the body. Wounds were noticeable especially on the ventral side of the posterior part of the body from which mucous was leaking. *Dugesia tigrina* individuals were contracted and regeneration lasted longer than in *Polycelis felina*. Body contractions and mucous excretion was more intense in individuals with heavier deformations. This is the defense mechanism of freshwater organisms to toxic chemical exposure (KALAFATIĆ *et al.* 2001). Even mortality was 25 % in *Dugesia tigrina.* An uneven distribution of pigment was remarkable. In *Dugesia tigrina* an accumulation of pigment could be noticed, especially on the anterior part of the body. In *Polycelis felina* pigmentation was unevenly distributed.

In conclusion, irradiation with UV-light of 253.5 nm wavelength for 20, 25 and 30 minute exposures did not cause damage to DNA or if it did cause damage, it was successfully and quickly repaired. This kind of observation was found in another simple aquatic organism *Hydra oligactis* Pallas (KALAFATIĆ *et al.* 2003).

The most intense cytological and histological changes were seen during the first two days after irradiation. As a result of body contractions in *Dugesia tigrina,* the lumen of intestines was reduced as compared to the control. Damage to the mucous layer and epidermal cells, as well as to rhabdites, could be seen in almost all the treated planarians. Rhabdites were not uniformly distributed in epidermis. Wounds were closed successfully in the period of recovery, during the first days after irradiation with UV-rays. In the wound areas reticular cells were present in greater numbers, performing phagocytosis of damaged cells and in this way prepared the damaged body parts for further regeneration, continued by the action of neoblasts. On the second day after UV exposure the number of neoblasts slightly increased and on the third day their number increased even more. Neoblasts successfully regenerated the damaged cells and tissues. On the fifth day of regeneration after irradiation, the cyto-histological structure of planarians of each species was similar to the control. Regenerated areas of the body were pale. They were built mostly of epidermal cells. Rhabdites were scarce in the regenerated areas of the planarian body. In the later course of regeneration the treated planarians did not differ from the control neither by cyto-histological nor by morphologica l

structure. DNA absorbs UV-B (315–280 nm), also called Medium Wave light, and the absorbed energy can break bonds in the DNA. Most of the DNAbreakages are repaired by proteins present in the nucleus but unrepaired genetic damage of the DNA can lead to serious changes in organism structure, cytology and morphology as is shown by this study. The penetration of increased amounts of UV-B light has caused great concern over the health of marine plankton that densely populate the top 2 meters of ocean water (TEVINI 1993) this can also be applied to freshwater organisms such as planarians which inhabit shallow parts of rivers and ponds.

Conclusion

On the basis of the performed experiments it can be concluded that individuals of *Polycelis felina*, which contain more pigment, were more resistant to UV-rays showing less change and faster recovery than individuals of *Dugesia tigrina.* Longer exposure to UV light can lead to serious damage to health of lower organisms as they do in higher organisms because UV light directly damages the DNA of living organisms regardless of their evolutionary complexity.

Pigmentation is one of the most important and evolutionary oldest mechanism of protection against damaging UV irradiation which is found across a broad range of animal phyla as a first line of defense against UV irradiation's damaging effects to organisms.

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References

FRANJEVIC D., KRAJNA A., KALAFATIC M., LJUBESIC N. 2000. The effects of copper upon the planarian *Polycelis felina* (Daly.). Period. biol. **102**: 283-287.

GRANT W. B. 2002. An estimate of premature cancer mortality in the US due to inadequate doses of solar ultraviolet-B radiation. Cancer **94**: 1867-1875.

KALAFATIC M., TABORSAK S. 1998. Effects of chromium upon neoblast division in the regenerates of *Polycelis felina*. Biologia (Bratislava) **53**: 321-325.

KALAFATIC M., TOMASKOVIC I. 1999. Toxic effects of aluminium in neutral and acidic media on the planarian *Polycelis felina*. Biologia (Bratislava) **54**: 711-716.

KALAFATIC M., TOMASKOVIC I. 2000. Nickel toxicity on the *Polycelis felina* (Daly.) species on the laboratory conditions. Period. biol. **102**: 289-292.

KALAFATIC M., KOVACEVIC G., ZUPAN I., FRANJEVIC D., MILIC-STRKALJ I., TOMASKVIC I. 2001. Toxic effects of chlorotolurone on the planarian *Polycelis felina* Daly. Period. biol. **103**: 263-266.

KALAFATIC M., KOVACEVIC G., ZUPAN I., FRANJEVIC D. 2003. Effect of Repeated UV-irradiation on *Hydra oligactis* Pallas. Period. biol. **105**: 171-175.

KOPJAR N., KALAFATIC M., BESENDORFER V. 1997. Mitotic and chromosomal disturbances in the planarian *Polycelis felina* caused by manganese. Biologia (Bratislava) **52**: 469-474.

KOPJAR N., KALAFATIC M., ŽNIDARIC D. 1994. Combined effects of Gamacide 20 and UV irradiation on green hydra (*Hydra viridissima* Pallas). Biologia (Bratislava) **49**: 371-375.

MATSUMURA Y., ANANTHASWAMY H. N. 2004. Toxic effects of UV radiation on the skin. Toxicol. Appl. Pharmacol. **195**: 298-308.

MILIC-STRKALJ I., KALAFATIC M. 1997. Effects of the herbicide Dicuran 500 FL upon the mitosis of the planarian *Polycelis felina*. Period. biol. **99**: 453-574.

RAFFA R. B., DASRATH C. S., BROWN D. R. 2003. Disruption of a drug-induced choice behavior by UV light. Behav. Pharmacol. **14**: 569-571.

RAFFA R. B., MARTLEY A. F. 2005. Amphetamine-induced increase in planarian locomotor activity and block by UV light. Brain Res. **1031**: 138-140.

SALO E. 2006. The power of regeneration and the stem-cell kingdom: freshwater planarians (Platyhelminthes) Bioessays. **28**: 546-559.

TEVINI M. 1993. UV-B Radiation and Ozone Depletion: Effects on humans, animals, plants, microorganisms and materials Lewis Pub. Boca Raton.

VAN DER HELM, P. J., KLINK E. C., LOHMAN P. H., EEKEN J. C. 1997. The repair of UV–induced cyclobutane pyrimidine dimers in the individual genes Gart, Notch and white from isolated brain tissue of *Drosophila melanogaster*. Mut. Res. **383**: 113-124.

VILLAR D., LI M. H., SCHAEFFER D. J. 1993. Toxicity of organophosphoshorus pesticides to *Dugesia dorotocephala.* Bull. Environ. Contam. Toxicol **51**: 80-87.