TOXICITY OF DINITROPHENYL DERIVATIVES USED AS PESTICIDES AND THEIR ENVIRONMENTAL IMPACT

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Several dinitrophenyl ethers such as 2,4-dinitroanisol, 2,4-dinitrophenetol, 2,4-dinitro-1-(octadecyloxy)benzene, 3-(2,4-dinitrophenoxy)propane-1,2-diol, as well as peptidoconjugates like 2,4-dinitro-glutathione or other similar compounds have been synthesized and tested within the germination experiments comparatively to the classical dinitrophenols, dinitrophenol pesticides, and sodium azide. Low concentrations (as low as 10^{-4} M) of both ethers and phenol derivatives inhibited seed germination, most probably by blocking oxidative phosphorylation. The toxicity mechanism of these pesticides, pesticide-like compounds and metabolic inhibitors was discussed in direct relationship with their infrared absorbance and fluorescence quenching. Thus, all of them have a significant absorbance at about 6000 cm^{-1} in IR, corresponding to ΔG of ATP formation and quench the fluorescence of tryptophan and other biological compounds.

***Key words**: pesticide toxicity, dinitrophenyl ethers, dinirophenols, wheat germination.*

Dinitrophenols and dinitrophenyl derivatives are compounds with multiple biological actions, being used as insecticides, ovicides, acaricides, fungicides and herbicides [*1-3*]. However, the dinitrophenol pesticides could be harmful to the environment, so that the toxicity level and mechanism should be investigated. Their action is in principle based on blocking the oxidative phosphorylation reactions and on inhibiting the formation of adenozin-triphosphoric acid starting from adenozin-diphosphoric acid [*4*]. Because the existing data are inconclusive and do not support a precise action mechanism of dintrophenyl derivatives on living organisms, it was necessary to synthesize some dinitrophenols and dinitrophenyl ethers whose biological activity should be tested.

The purpose of this paper is to compare the action of low concentrations of some dinitrophenols and dinitrophenyl ethers with that of some known uncouplers and metabolic inhibitors [*5*]. Because germination experiments are easy, cheap, fast and spectacular, the testing of the action of some action of some known and newly synthesized substances on living organisms will be performed using germinating cereal seeds [*3*]. The possible mechanism of toxicity of these chemicals and pesticides are discussed in the light of the biostructural theory by Eugen Macovschi as well as the chemiosmotic theory by Peter Mitchell [*6-8*].

MATERIALS AND METHODS

Biological material. The wheat samples (*Triticum aestivum*), Henika variety, were taken from the Agricultural Research Station in Suceava. The 1000 seeds weighed 37,2 g and had a residual humidity of 12%.

Chemical reagents. The reagents used were of analytic purity (Merck, Sigma, Chimopar) and the solution and the water slurries were prepared using bidistilled water. A series of dinitrophenol ethers were synthesized, starting from dinitrochlorobenzene, with variants of the methods described in literature [3]. Thus, dinitroderivatives such as 2,4-dinitrophenetol, 2,4-dinitroanisol, 3-(2,4-dinitrophenoxy)propane-1,2-diol, 2,4-dinitrophenyl-glutathione and 2,4-dinitro-1-(octadecycloxy)benzene were synthesized.

Several solutions of dinitrophenyl ethers and dinitrophenols with the concentrations in the range from 10^{-4} M to 10^{-3} M were prepared. A blank with bidistilled water was also carried out.

Equipment. The chemical syntheses were carried out using the organic chemistry lab equipment of the Chemistry Department of "Al. I. Cuza" University of Iasi. The experiments and the germination determinations were performed in Petri dishes, on double Watmann no. 1 filter paper at room temperature. The separation and purification of the compounds obtained were carried out using thin layer chromatography on silica gel (Kieselgel $60F_{254}$, Merck) and on silica gel column. The infrared spectra were taken on a Jasco FT/IR660 Plus Fourier spectrometer in the range from 0 to 15000 cm^{-1}. The melting points and the elemental composition were also determined and ^{1}H-RMN (Bruker Advance DRx400, 400 MHz) spectra and mass spectra were performed using a Vestec 601 mass spectrometer.

Procedure. The germination parameters were measured according to ISTA recommendations (Seed Science and Technology, 1993), however we worked also with lots of 50 seeds which were laid to germinate on filter paper, in Petri dishes, in three repetitions. The first count took place after three days (energy of germination, EG), the second after 7 days (germination rate, GR). The germinated, abnormal and dead seeds as well as the resulting plantlets were counted.

The treatment lasted for an hour, then the seeds were laid as uniformly as possible in Petri dishes, on double filter paper, together with the treatment solution. The seeds with a visible root were considered germinated. The seeds were watered daily with 5 ml of bidistilled water. The plantlets were cut at the level of the seeds 7 days after, measured and weighed (height, H, in cm and mass, m, in grams).

Statistics. The results were processed using the Tukey test [8].

RESULTS AND DISCUSSION

Some dinitrophenyl ethers and dinitrophenols were synthesized and characterized from the chemical point of view. Previously, we demonstrated that the inhibition of wheat germination was not significant below 10^{-3} M when used 2,4-dinitroanisol. However, we show in this paper a significant toxicity of low concentrations of other dinitrophenyl ethers and phenols (*figures 1 and 2; table 1 and 2*). Thus, 2,4-dinitrophenol at 10^{-4} M concentration strongly reduced the total height of the plantlets from 631.7 cm to 369.0 cm (by 42%), whereas the same concentration of sodium azide diminished the same parameter by 31.7%. Another experiment showed that 3-(2,4-dinitrophenoxy)propane-1,2-diol at $3x10^{-4}$ M succeeded to inhibit the wheat germination and to reduce the total height of the plantlets from 625.7 cm to 584.3 cm (by 6.6%). Therefore, low concentrations of both ethers and phenol derivatives inhibited seed germination even below 10^{-4} M, most probably by blocking oxidative phosphorylation. Taking into consideration the chemical analyses of investigated the compounds, the FT-IR spectra (*figure 3*), the melting points and the elemental analyses of the chromatographically purified compounds confirmed their structures. Thus, the dinitrochlorobenzene signals at 1962.2 cm^{-1} and 1706.7 cm^{-1}, respectively, disappeared and new bands appeared in the dinitrophenyl derivatives spectra at 1280.5 cm^{-1} and 1000.9 cm^{-1}. The two nitro groups of the synthesized precursors or the newly synthesized compounds presented a characteristic and intense signal at 1530 cm^{-1}- 1540 cm^{-1}.

The toxicity mechanism of these pesticides, pesticide-like compounds and metabolic inhibitors may be discussed in direct relationship with their infrared absorbance (*figure 3*) and fluorescence quenching (not shown). Thus, all of them have a significant absorbance at about 6000 cm^{-1} in IR, corresponding to ΔG of ATP formation and quench the fluorescence of tryptophan and other biological compounds (*figure 3*).

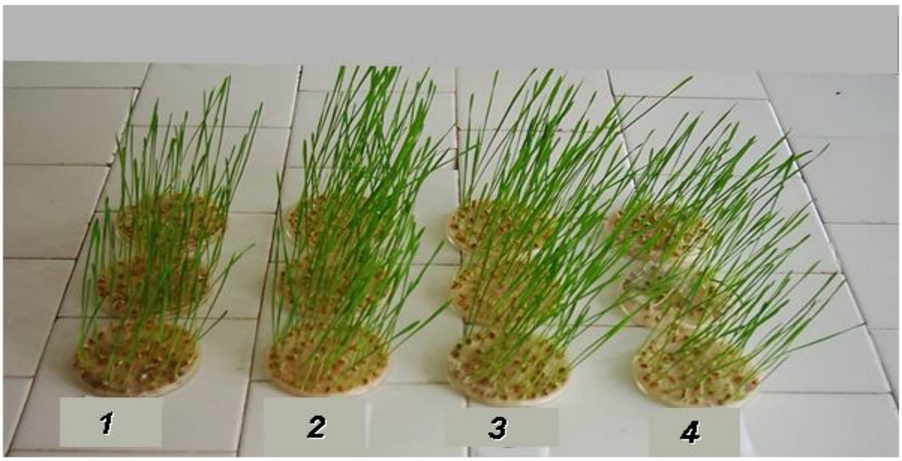

Figure 1. **The effect of low concentrations of 2,4-dinitrophenol (DNP) and sodium azide (NaN_3) on wheat seed germination. 1. DNP, $5x10^{-4}$ M; 2. Blank, H_2O; 3. NaN_3, 10^{-4} M; 4. NaN_3, $5x10^{-4}$ M; contact time: 7 days.**

A significant difference was also calculated with the aid of Tukey test between the blank and the sample treated with 3-(2,4-dinitrophenoxy)propane-1,2-diol, with a concentration of $3x10^{-4}$ M. Another significant difference was noticed between the treatment with a concentration of $3x10^{-4}$ M and that of the DNP at the same concentration (*figure 2*).

Therefore, even at very low concentrations, dinitrophenyl derivatives proved to have a toxic effect, although they possess no free –OH groups able to determine an H^+ gradient through the biological membranes.

The results were processed using the Tukey test and the differences between the heights values of the lots treated with various dinitrophenyl derivatives proved to be significant. Results were discussed in the light of the biostructural theory by Eugen Macovschi and the chemiosmotic theory by Peter Mitchell.

Table 1

The toxicity of 2,4-dinitrophenol and sodium azide at low concentrations in a wheat seeds germination experiment

Treatment	Germination rate (G.R.)	Number of plantlets in a lot (N)	Total hight of plantlets of the lot (**H**, cm)	Plantlets size (**S**, cm)
DNP, $5x10^{-4}$ M	90%	38	369.0±28.4	9.8±0.9
Blank, H_2O	97%	46	631.7±64.6	13.8±0.1
NaN_3 , $1x10^{-4}$ M	95%	43	547.2±39.3	12.7±0.2
NaN_3 , $5x10^{-4}$ M	91%	38	416.6±39.3	11.14±0.6
D (Tukey test)	8	6	46.8	0.6

*) Average of three independent values.

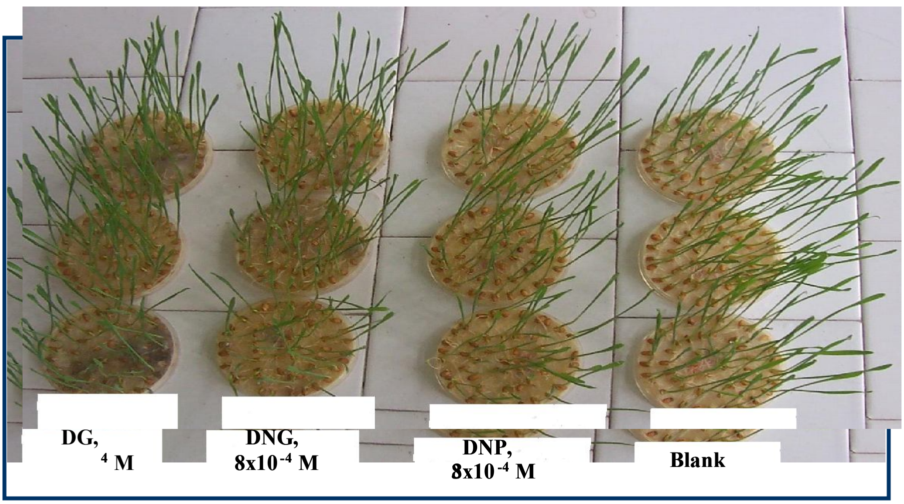

Figure 2 **The biological effect of low concentrations of 3-(2,4-dinitrophenoxy)-propane-1,2-diol (DNG) and 2,4-dinitrophenol on wheat germination.**

It was assumed that dinitrophenol dissociates releasing protons, which penetrate the mitochondrion, replacing to one translocated from the mitochondrion into the cytosol, preventing the formation of ATP. Previous research has indeed suggested that a pH [7,8] gradient interferes in the formation of ATP. In the case of of DNG, the –OH group, of the 2,4-dinitro-phenol, was replaced by the –

$OCH_2CH(OH)CH_2OH$ group. Because this compound, which does not have the possibility to easily release a proton, was proved to be toxic (Table 2), the mechanism of DNP should be different from the one mentioned in literature [7,8].

Tabelul 2

The inhibitory effect of 3-(2,4-dinitrophenoxy)propane-1,2-diol (DNG) and 2,4-dinitrophenol (DNP) on wheat germination and the growth of the resulted plantlets

Treatment *)	Germination rate (G.R.)	Number of plantlets in a lot (N)	Total hight of the plantlets in a lot (**H**, cm)	Plantlets size (**S**, cm)
DNG, $3x10^{-4}$ M	96%	46	584.8±33.8	13.2±0.8
DNG, $8x10^{-4}$ M	96%	46	565.4±40.0	13.0±0.6
DNP, $3x10^{-4}$ M	94%	42	507.3±29.1	12.3±0.1
Blank, H_2O	96%	46	625.7±43.7	13.7±0.4
D (Tukey test)	12	2	39.7	0.4

*) Average of three independent values.

Our studies take into consideration the fact that the pH modification could be a secondary phenomenon, being possible to transfer the energy of triplet states to the ADP molecule, which incorporates it as ATP. The transition from an excited state to a normal state leads to the release or absorption of a proton, depending on the acid or base character of the compound that is in an excited state.

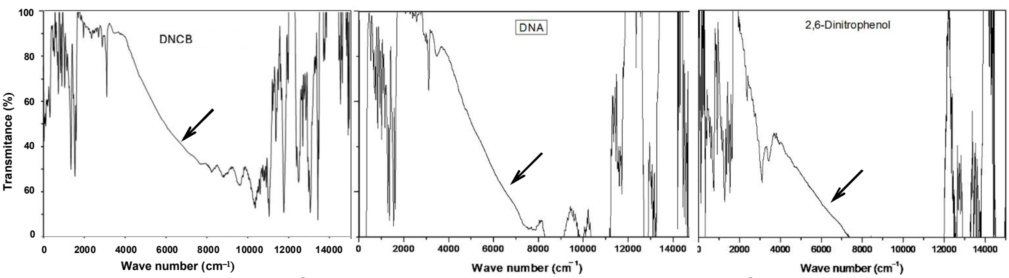

Figure 3 **FT-IR spectra of the precursor dinitrochlorbenzene (left), 2,4-dinitroanisol (middle), as well as 2,6-dinitrophenol (right). The high absorbance in infrared at about 6000 cm^{-1}, corresponding to the formation energy of ATP suggests a radiation-based mechanism of toxicity of these compounds.**

2,4-Dinitrophenols act as uncouplers of the breathing from oxidative phosphorylation, which results in an intensified oxygen consumption, without ATP synthesis. Dinitrophenols normally disturb ATP production within the cellular mitochondria, because the ATP is the molecule which stores and supplies energy for cellular activities [6]. Present theories, such as P. Mitchell's chemiosmotic theory, claim that, unlike the other enzymes in the mitochondrial respiratory chain, the ATP pumps protons from the intermembrane space towards the matrix. Thus, the energy that the other enzymes in the chain use to accumulate protons in the intermembrane space is recuperated. This energy is necessary for the ADP phosphorylation reaction with the mineral phosphate, in the presence of Mg ions, the reaction being endothermic and requires more than 31 kJ/mol.

Present research showed that it is possible for the dinitrophenyl ethers to act in a non-chemical way, probably through radical and triplet status formation, and that the proton translocation could be a secondary phenomenon in the process of oxidative phosphorylation. The action mechanism of the compounds investigated is concordant with Eugen Macovschi's well-known biostructural theory, but contradicts Peter Mitchell's chemiosmotic hypothesis.

CONCLUSIONS

New dinitrophenol derivatives were synthesized and characterized, some of them unknown in literature, as well as used to understand better the toxicity and biological action mechanism of dinitrophenol pesticides. The investigation of numerous findings supports the hypothesis that dinitrophenols cancel the ATP formation by hindering or destroying the excited electronic states. Besides, the excitation of organic molecules is connected with changes in their acidity and, therefore, a proton pump cannot explain ATP production. Further investigations using the new theoretical instruments should be undertaken to clarify these obscure aspects of dinitrophenols biological activity.

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