SECOND-HAND CLOTHES WASHED WITH DETERGENTS FOR CHILDREN'S CLOTHES: TOXICITY OF WATER-SOLUBLE RESIDUAL COMPOUNDS ACCORDING TO PHYTOTESTING AND HEALTH RISKS FOR CHILDREN

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Abstract. *The purpose of this study was to investigate the toxicity (according to the phytotest with garden cress) of water-soluble residual compounds from reusable clothes ("second hand"), provided that they were washed with detergents for children's underwear. Phytotesting with garden cress (Lepidium sativum L.) and statistical methods of processing the results were used in the study, the root length index and the phytotoxic effect of the solutions were calculated. Detergents for children's clothes, widely available in the retail network of Ukraine, were used for washing. It was established that the sensitive test indicators of garden cress to the water-soluble residual toxicants of the "second hand" clothing material are the length of the roots and the aerial part of the seedlings. Phytotoxic properties were noted for solutions obtained from the material without treatment and after washing with phosphonate-containing and phosphate-free products. The consequences of fabric treatment with a laundry conditioner after washing are ambiguous: the phytotoxic properties of the water-soluble residual compounds of phosphonate-containing powder were eliminated, but the phytotoxicity of the phosphate-free powder remained. Water-soluble residual compounds of household soap had a favorable effect on the growth of the roots of the test plant. Second-hand clothes washed with the specified detergents are potentially dangerous for the child's health, except for clothes washed with household soap, which eliminates the toxicity of the material and does not lead to the appearance of factors that are negative for health.*

Introduction. The declared value of the world trade in second-hand clothes is constantly increasing. The trade value of total exports rose from USD 0.75 billion (1990), to USD 1.53 billion (2001) and USD 4.2 billion (2018) (Lampel 2020). The low solvency of the citizens of Ukraine and the lack of provision of the population with basic wardrobe items determined the importance of second-hand clothing stores for people with low incomes (Kuchma 2010). According to unofficial statistics, 60-80% of Ukrainians buy clothes from second-hand stores (Bazik and Gayova 2019).

The reuse of clothes is one of the approaches of the concept of circular business models, which is widely used in the world (Mytsenko and Khadzhinov 2022; das Virgens et al. 2022) and allows a person and society as a whole to solve a number of environmental, economic and social problems (Filho et et al. 2019). Collected clothes can be: 1) reused as second hand; 2) rework (Kirichenko 2021). According to studies by Filho et al. (2019) textile reuse and recycling has environmental, economic and social benefits.

Along with this, new problems are emerging: environmental (accumulation of garbage in countries with a low level of socio-economic development), economic (decline of the textile industry) and social (deterioration of human health) (Kratik 2019; Kirichenko 2021). So, in reality, using used clothes does not solve environmental, economic and social problems.

In particular, there are risks to human health as a result of non-compliance with sanitary standards for processing clothes with formaldehyde (Mala 2017; Kratik 2019). High doses of formaldehyde can also be contained in new cotton items due to the peculiarities of the cotton fabric manufacturing technology (Herrero et al. 2022; Reitz et al. 2022). A safe and simple practice to remove formaldehyde from such items is to wash the garment before use (Herrero et al. 2022). Since children are the most sensitive to toxicants (Au 2002), special attention should be paid to children's clothing.

Both chemical and biological methods are used to assess environmental pollution, the toxicity of various compounds, and their impact on living organisms. One of the available and easy-to-perform biological methods is phytotesting (Smykun and Furman 2008; Torgashkova et al. 2018; Revathi and Sheena 2019; Calvo-Olvera et al. 2021; Bernegossi et al. 2022). Garden cress (*Lepidium sativum*) is one of the sensitive plant models in this test (Galli et al. 2019; Bożym 2020; Tkachuk and Okulovych, 2021; Tkachuk et al. 2022). Therefore, the purpose of this work was to study the toxicity (according to the phytotest with garden cress) of reusable clothes ("second hand") with different options for washing by detergents for children's clothes and to assess the risks to children's health when using such clothes.

Materials and methods. *Selection of "second hand" clothing samples and their preparation for research.*

Children's clothes made of 100% cotton (T-shirts) of white color was chosen for the study, which was purchased in the well-known chain of "second hand" stores in Chernihiv (Ukraine) in the amount of 3 pieces. From each T-shirt, 6 samples of material measuring 10 × 10 cm (18 samples in total) were cut. Of these, 3 samples (one from different T-shirts) were left untreated (option 2), and the other samples were washed by hand using various detergents, air-dried and ironed. Washing was carried out with the amount of detergent recommended by the manufacturer.

The following washing options of fabric "second hand" were used: option 3 – phosphonate-containing washing powder for children's clothes; option 4 - phosphonate-containing washing powder for children's clothes with subsequent treatment with conditioner for children's clothes; option 5 – phosphate-free washing powder for children's underwear; option 6 – phosphate-free washing powder for children's underwear with subsequent processing by conditioner for children's clothes; option 7 - household soap, 72%. Detergents widely available in the retail network of Ukraine were used. In order to prevent accusations of advertising or anti-advertising of laundry detergents, we do not list their trade names.

According to manufacturers, the composition of detergents is as follows:

• phosphonate-containing washing powder for children's clothes: 5-10% anionic surfactants, oxygen brighteners, zeolites; < 5% nonionic surfactants, cationic surfactants, phosphonates, soap, polycarboxylates; additionally: enzymes, optical brighteners, perfume composition.

• phosphate-free washing powder for children's underwear: > 30% sodium chloride; 15-30% sodium carbonate; 5-15% silicate; < 5% sodium percarbonate; soap; anionic surfactants; tetraacetylethylenediamine (TAED); perfumes.

• conditioner for children's clothes: < 5% cationic surfactants, < 5% nonionic surfactants, flavoring additive (hexyl cinnamal), preservative (benzisothiazolinone, methylisothiazolinone), aloe vera leaf juice.

• household soap (72 %): sodium salts of fatty acids of tropical vegetable oils and animal fats, water, glycerin, disodium ethylenediaminetetraacetate (disodium EDTA), antioxidant.

After drying in the fresh air and ironing, each sample was immersed in distilled water (100 ml) for 2 hours at a temperature of 24 ± 1 ºC to dissolve water-soluble residual compounds from the material in the water. The resulting solutions (aqueous extracts) were used in phytotoxicity studies.

Study of the toxicity of "second-hand" clothing samples by phytotesting with L. sativum.

Garden cress (*L. sativum*) produced in the Czech Republic (MoravoSeed), packaged by private enterprise "Scientific and production firm "Tiras", batch No. 69088-01, which, according to the information on the package, corresponds to State Standard of Ukraine 6006:2008 was used as a test plant. Garden cress seeds were placed on filter paper located at the bottom of self-made containers made of food plastic and moistened with the appropriate test solution (see the previous section). In the control, seeds were germinated on filter paper while watering with distilled water (option 1). The number of seeds per container is 10 pieces, the repetition of control and each experiment is threefold. Seed germination took place in the dark at a temperature of 24 ± 1 ºC. On the 3rd day, germination energy was determined, on the 5th day - germination and the length of the aerial part and roots.

Phytotoxic indices were calculated - root length index (RLI) and phytotoxic effect of solutions (PhTE) according to formulas (1) and (2), respectively.

$$RLI = \frac{L_T(i) - L_C}{L_C}, \quad (1)$$

where $L_T(i)$ and L_C are the average length of roots in the test (i) and in the control, respectively. Phytotoxicity was determined using the following scale (Bagur-González et al. 2011; Mtisi and Gwenzi 2019):

weak: $-0.25 \leq RLI < 0$;

average: $-0.5 \leq RLI < -0.25$;

high: $-0.75 \leq RLI < -0.5$;

extreme: $-1 \leq RLI < -0.75$.

$$PhTE = \left(\frac{L_C - L_T(i)}{L_C}\right) \times 100, \quad (2)$$

where $L_T(i)$ and L_C are the average length of roots in the test (i) and in the control, respectively (Baghdasaryan 2005).

Statistical analysis of experimental data.

The statistical module of the Microsoft Office Excel 2010 program was used for statistical processing of the obtained data. Descriptive statistics methods were used - calculation of the arithmetic mean value (M) and the standard error of the arithmetic mean value (m). The number of observations (n) for biometric indicators of seedlings was from 25 to 30 (depending on the number of seeds that germinated in each variant of the experiment). The significance of the differences between the control and the experiment was assessed by the Student's significance test (t). The statistical significance of differences in germination energy and seed germination (n = 3) was assessed using the χ^2 test using the Past 4.03 software (Hammer et al., 2001). A 95% probability of differences ($p \leq 0.05$) was considered statistically significant.

Results and discussion. Garden cress is a toxicant-sensitive plant that is used as a model test system in various toxicological studies (Galli et al. 2019; Bożym 2020; Tkachuk et al. 2022). Therefore, we used it to evaluate the toxicity of aqueous solutions obtained in the process of preparing material samples from "second-hand" children's clothing. The obtained results are presented below.

Test indicators of L. sativum

The results of the study of garden cress test indicators are presented in Table 1.

Table 1

Test indicators of *L. sativum*

Research option	The energy of germination, % (M ± m; n = 3)	Germination, % (M ± m; n = 3)	Length, mm	
			Roots (M ± m; n)	Above ground part (M ± m; n)
1	97.0 ± 3.0	97.0 ± 3.0	47.9 ± 3.1; 29	34.9 ± 1.8; 29
2	93.0 ± 3.0	97.0 ± 3.0	49.0 ± 3.9; 28	30.1 ± 1.4*; 28
3	97.0 ± 3.0	97.0 ± 3.0	26.6 ± 2.2*; 29	29.2 ± 1.4*; 29
4	97.0 ± 3.0	100	43.3 ± 3.3; 29	30.9 ± 1.3; 29
5	100	100	36.0 ± 2.8*; 30	29.7 ± 1.3*; 30
6	83.0 ± 3.0	97.0 ± 3.0	39.5 ± 4.2*; 25	35.4 ± 2.4; 25
7	97.0 ± 3.0	97.0 ± 3.0	62.4 ± 4.2*; 29	34.6 ± 1.8; 29

Note: * - differences from the control are significant at $p \leq 0.05$ (t_{st}=2.01-2.68-3.50); option 1 – the control (filter paper and distilled water); option 2 – the filter paper and aqueous extract of the fabric "second hand" without washing; option 3 – the filter paper and aqueous extract of the fabric "second hand" with washing with phosphonate-containing washing powder for children's clothes; option 4 – the filter paper and aqueous extract of the fabric "second hand" with washing with phosphonate-containing washing powder for children's clothes with subsequent treatment with conditioner for children's clothes; option 5 – the filter paper and aqueous extract of the fabric "second hand" with washing with phosphate-free washing powder for children's underwear; option 6 – the filter paper and aqueous extract of the fabric "second hand" with washing with phosphate-free washing powder for children's underwear with subsequent treatment with conditioner for children's clothes; option 7 – the filter paper and aqueous extract of the fabric "second hand" with washing with household soap (72%)

It was established that the tested solutions did not have a toxic effect on germination energy and seed germination (see Table 1). However, the length of the roots and the aerial part of the seedlings turned out to be sensitive indicators. Thus, it was established that the solution obtained during the polishing of the samples of option 2 (second-hand clothing material without washing) had a negative effect on the length of the above ground part: the indicator statistically significantly decreased compared to the control by 1.2 times, but the length of the roots was within the control limits (see Table 1).

The results of the study of phytotoxicity of solutions obtained after washing materials with synthetic detergents - phosphonate-containing powder for children's clothes (option 3) and phosphate-free powder for children's clothes (option 5) deserve attention. In both of these options, a significant decrease was recorded compared to the control in the length of the roots (by 1.8 times and 1.3 times, respectively) and the aerial part (by 1.2 times) of garden cress seedlings (see Table 1). At the same time, the roots of the test plants in variant 3 (phosphonate-containing powder) turned out to be darker than in the control variant. It is possible that the toxicity in options 3 and 5 is related to both the influence of the final amounts of formaldehyde and the constituent compounds of the detergents.

The result of fabric treatment with laundry conditioner turned out to be ambiguous. Thus, in the variant 4, the phytotoxic properties of the solution were not recorded, and in the variant 6, a statistically significant decrease was noted compared to the control in root length (by 1.2 times) (see Table 1). So, in the case of phosphonate-containing powder, additional rinsing of clothes after washing with conditioner for children's clothes eliminates the phytotoxic properties of its residual amounts, which cannot be said about phosphate-free powder.

The solution obtained from "second-hand" clothing material after washing it with household soap (variant 7) reliably stimulated the growth of roots - the rate was 1.3 times higher than in the control (see Table 1). So, in this case, phytotoxic properties were not recorded; the amount and composition of residual compounds of household soap proved to be favorable for the growth of the roots of the test plant.

Phytotoxic indices

Based on the results of the root length study, the phytotoxic indexes listed in Table 2 were calculated.

Table 2

Interpretation of the data obtained in the experiment

Research option	RLI	PhTE	Interpretation of phytotest results	Comments
1	Calculation is not expected	Calculation is not expected	No toxicity	No inhibition of growth
2	0.023	-0.023	No toxicity	No inhibition of growth
3	-0.445	0.445	Average toxicity	Average (40 %) growth inhibition
4	-0.096	0.096	Weak toxicity	Weak (10 %) inhibition of growth
5	-0.248	0.248	Weak toxicity	Weak (25 %) inhibition of growth
6	-0.175	-0.175	Weak toxicity	Weak (18 %) inhibition of growth
7	0.303	-0.303	No toxicity	No inhibition of growth

Note: option 1 – the control (filter paper and distilled water); option 2 – the filter paper and aqueous extract of the fabric "second hand" without washing; option 3 – the filter paper and aqueous extract

of the fabric “second hand” with washing with phosphonate-containing washing powder for children’s clothes; option 4 – the filter paper and aqueous extract of the fabric “second hand” with washing with phosphonate-containing washing powder for children’s clothes with subsequent treatment with conditioner for children’s clothes; option 5 – the filter paper and aqueous extract of the fabric “second hand” with washing with phosphate-free washing powder for children’s underwear; option 6 – the filter paper and aqueous extract of the fabric “second hand” with washing with phosphate-free washing powder for children’s underwear with subsequent treatment with conditioner for children’s clothes; option 7 – the filter paper and aqueous extract of the fabric “second hand” with washing with household soap (72%)

According to the calculated indices, aqueous solutions 2 (fabric without washing) and 7 (fabric after washing with household soap) do not have toxic properties (see Table 2). An aqueous solution of second-hand clothing fabric after washing in a phosphonate-containing powder showed an average level of toxicity, and a weak level of toxicity in a phosphate-free powder. Phytotoxic indices of aqueous solutions from clothes after additional treatment with conditioner for children’s clothes characterize them as weakly toxic.

According to calculated indexes that take into account the length of garden cress roots, “second hand” clothes without washing are non-toxic, but the indicator of the length of the above-ground part shows slight phytotoxicity (see Table 1-2). Therefore, wearing such clothes without washing is dangerous because of the potential negative impact on health. The potential health risks associated with formaldehyde used to treat such items depend on the routes of exposure (such as inhalation or skin contact), the concentration of formaldehyde, and the duration of exposure. Inhaling formaldehyde can cause effects such as nausea, asthma exacerbations, and cellular changes that can lead to the development of tumors. Chronic inhalation exposure to formaldehyde can cause cancer (GAO-10-875 2010; Rovira et al. 2016).

However, formaldehyde in clothing is of greatest concern because allergic contact dermatitis occurs as a result of exposure to it on the skin. The formation of eczema, allergic contact dermatitis, affects the immune system and causes reactions characterized by rashes, blisters and peeling of dry skin, itching or burning. Another possible health effect from exposure to formaldehyde on the skin - irritant contact dermatitis - is also a form of eczema and has similar symptoms; however, the condition does not affect the immune system.

Avoiding clothing containing formaldehyde is usually effective in preventing allergic and irritant contact dermatitis and relieving symptoms, but this can be difficult to do because clothing labels do not list items that have been treated or contain formaldehyde. Washing clothes before wearing often reduces formaldehyde levels, but is not always successful. In some cases, avoiding or alleviating allergic contact dermatitis requires more drastic measures, such as taking medications with potentially serious side effects.

Finally, consumers may also be exposed to formaldehyde on their skin through the use of certain cosmetics, including skin care products, shampoos, and sunscreens that contain formaldehyde (GAO-10-875 2010). Second-hand clothes treated with formaldehyde, which has high bactericidal properties, can negatively affect the health of people - both consumers and workers of second hand clothing stores (Kratik 2019).

The obtained results indicate the necessity of washing second-hand clothes. At the same time, washing with synthetic detergents (both phosphonate-containing and phosphate-free) poses a threat to the health of children - residual compounds ensure medium and low toxicity of clothes. In

particular, phosphonates, which are esters and salts of phosphonic acids and are being developed as an alternative to phosphates in laundry detergents, can cause a dermatological reaction (Dirty laundry… 2016).

The use of phosphonates in powders is a step on the way to reducing the content of phosphates in detergents, however, they are worse rinsed, and still lead to an increase in the content of phosphorus compounds in wastewater, and, therefore, to the deterioration of the ecological condition of water bodies where such wastewater enters. Among the compounds that can affect the health of children, the composition of the used phosphonate-containing product also includes: 1) zeolites, which have an increased content of silicates, which causes degreasing of the skin, as well as pollution of water bodies with aluminum; 2) optical bleaches (have a reflective effect and create the illusion of "shining" laundry, and do not affect the actual cleanliness of the laundry; they practically do not rinse off - their task is to remain on the fabric), which can cause contact dermatitis (Dirty laundry… 2016).

The composition of phosphonate-containing and phosphate-free products includes aromas that cause itching of the body, hands, eyes, contribute to the development of allergies and asthma. Also, both types of washing powders contain surface-active substances, the impact of which on health is manifested in impaired immunity, development of allergies, damage to the brain, liver, kidneys, and lungs (Dirty laundry… 2016).

There is evidence that surfactants can affect the human body for a long time due to the property of gradual accumulation in the brain, liver, heart, and subcutaneous tissue (Yuan et al. 2014). That is, they affect the human body as a whole, and not only at the skin level. The problem is also complicated by the inability of the vast majority of sewage treatment plants in our country to qualitatively remove surfactants and, as a result, their gradual accumulation in the environment (Frolova et al. 2019).

TAED has very low toxicity by all considered routes of exposure, practically does not irritate the skin and eyes, there is no evidence of sensitizing potential when in contact with the skin (Human & Environmental Risk Assessment... 2002).

It is possible to recommend, after washing in the investigated powders, additional treatment with a conditioner for children's clothes, which contains components of plant origin, which, according to the results, reduces the toxicity of the material, and, therefore, the risks of impact on health. However, the studied conditioner for children's clothes contains surfactants, as well as: 1) hexyl cinnamaldehyde, which is included in the group of compounds that cause skin irritation and are skin sensitizers (allergens) (Amyl and hexyl cinnamaldehyde... 2016); 2) benzisothiazolinone, which causes skin irritation and can cause a skin allergic reaction, causes serious eye damage (Benzisothiazolinone and its salts... 2019); 3) methylthiazolinone, which is a pesticide and is used to control slime-forming bacteria, fungi, algae in pulp and paper mills, water cooling systems, oilfield operations, industrial process water and air purification systems and is included in adhesives, coatings, fuels, processing fluids metals, resin emulsions, paints and various other special industrial products as a preservative (Methylisothiazolinone... 1998).

Methylthiazolinone is also used to control mold growth on wood products. In studies using laboratory animals, methylisothiazolinone was found to exhibit moderate acute toxicity by the oral and inhalation routes; has high acute toxicity when applied to the skin or eyes. In subchronic studies, the most significant toxicological effect was microscopic damage to the turbinates due to inhalation exposure. Developmental and chronic feeding/carcinogenicity studies in rats yielded no significant effects, and the US Environmental Protection Agency has classified methylisothiazolinone as a Group

D chemical not classified as carcinogenic to humans. The results of the mutagenicity study were questionable (Methylisothiazolinone... 1998).

Second-hand clothes washed with household soap will not have a negative impact on the child's health, because, according to the results of the study, the material does not show phytotoxicity. The composition of household soap includes EDTA. EDTA can contribute to aquatic toxicity at low concentrations, and its release into natural waters should be minimized where possible (Sillanpää 1997; Oviedo and Rodrígues 2003). Lanigan and Yamarik (2002), based on an analysis of publications, note that EDTA and its salts have been evaluated for their potential ability to cause chromosomal aberrations, semi-lethal processes, crossovers, direct mutations, replicative DNA synthesis, DNA strand breaks, dominant lethal processes, metabolic inhibition, sister exchange chromatids with mostly negative results (Lanigan and Yamarik 2002). So, in general, the best approach for removing the toxicity of second-hand material is washing with laundry soap.

Conclusion. Thus, the length of the roots and the above ground part of the seedlings turned out to be sensitive test indicators of garden cress. Phytotoxic properties were noted for solutions obtained from the material without treatment and after washing with phosphonate-containing and phosphate-free products. The consequences of fabric treatment with a laundry conditioner after washing are ambiguous: the phytotoxic properties of the phosphonate-containing powder were eliminated, but the phytotoxicity of the phosphate-free powder remained. The water-soluble residual compounds of the household soap had a beneficial effect on the growth of the roots of the test plant. The solutions obtained after processing the investigated samples of second-hand clothing material with detergents can be arranged in the following order of decreasing toxicity: washing powder for children's clothes (phosphonate-containing) > washing powder for children's clothes (phosphate-free) > washing powder for children's clothes (phosphate-free) + conditioner for children's clothes > washing powder for children's clothes (phosphonate) + conditioner for children's clothes > household soap. Second-hand clothes washed with the specified detergents are potentially dangerous for the child's health, except for clothes washed with household soap, which eliminates the toxicity of the material and does not lead to the appearance of factors that are negative for health.

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