Health-risk assessment based on an additive to paints made from isobutyric aldehyde condensation products

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Abstract. Solvents are primarily used for making protective coatings. Considering their chemical nature, there are a great variety of coatings, including those based on liquid hydrocarbons and organic chloroderivatives. These products are a serious load to the environment because of their physicochemical properties, therefore, they have for some time been replaced with more-environmentally friendly, new generation products. One of them is the hydroxyester HE-1: made from isobutyric aldehyde condensation products, it is an alternative to those coalescents for paints and varnishes which are intended to be replaced or their use restricted.

The results of selected toxicological tests relating to the human health risk effect of the hydroxyester HE-1 – environmentally-friendly additive to paints and varnishes are presented. The test results indicate that HE-1 causes skin irritation in rabbit only when used at its maximum concentrations. No lesions in the cornea or iris were observed in any of the test rabbits after the application of the hydroxyester HE-1. In the mutagenic effect test of HE-1 on the bacteria *Salmonella typhimurium,* the result was negative. Based on the test results, it was found that the hydroxyester HE-1 may only have a human health risk effect when used at its maximum concentrations.

1 Introduction

Nearly one-half of the volume of globally used solvents is in the production of protective coatings. These solvents are volatile products, made by the processing of natural gas and petroleum. They include liquid hydrocarbons and organic chloroderivatives which are a serious load to the environment [1, 2]. This is regulated in the Decopaint Directive (2004/42/EC) which came into force on 1 January 2007 [3].

It relates to the reduction of emissions of solvents from decopaints and automotive varnishes, other than those covered by the limitations of Directive No. 1999/13/EC concerning solvent emissions [4]. According to the Decopaint Directive, any substance having its initial boiling point at 250°C or lower, as measured at a pressure of 101.3 kPa, is a Volatile Organic Compound (VOC).

Lower VOC levels in paints can be obtained by developing paint formulations which contain coalescents not classified as VOC, or which contain coalescents and glycols classified as VOC in amounts not higher than the limits referred to in the applicable laws [5].

Phthalates were the prior choice as additives to paints and varnishes. Considering their physicochemical properties, there are two groups of phhthalates: high-molecular and low-molecular products. The high-molecular phthalates, which include di-isononyl phthalate (DINP) and di-isodecyl phthalate (DIDP), represent 80% of the consumption of phthalates in Europe alone. The low-molecular phthalates include dibutyl phthalate (DBP), benzyl-butyl phthalate (BBP), and di-2-ethyl-hexyl phthalate (DEHP), which are classified as products with high human health risk [1]. Based on animal tests, it was found that the low-molecular phthalates are toxic, therefore, they may not be used for manufacturing toys, articles for children, cosmetics, and medical devices [6].

The adverse effect of phthalates on human health has been confirmed in a number of other research reports [2,7,8].

Most of the conventional coalescents, which compete with one another on the market, have their boiling points below 250˚C – the criterion for being classified as VOC – thus falling into the category of VOC with applicable quantitative limitations. Some new products on the market have their boiling points above the criterion for VOC (Table 1). The products with boiling points below 250°C are approved for use on the condition that their total VOC emissions are not higher than the limits set out in the Decopaint Directive [3].

The withdrawals in the sector of coalescents for paints and varnishes or products with limited use can be replaced, as an alternative, with the hydroxyester HE-1 (Fig. 1).

The aim of the study was to determine a human health risk effect of hydroxyester HE-1 and another advanced additives to paints and varnishe.

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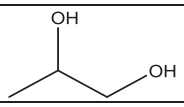
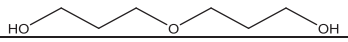
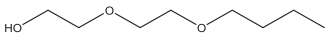
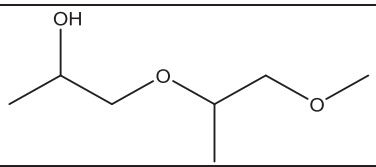
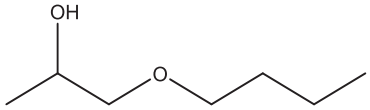
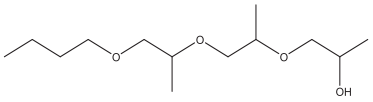
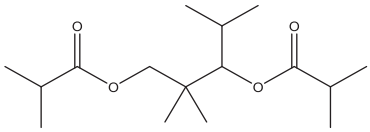
Table 1. Structural formulae and boiling temperatures of advanced additives to paints and varnishes [5,9].

Compound	CAS Number	Boiling point [^{o}C]	Structural formula
Propane-1,2-diol	57-55-6	187.4	
Oxydipropanol	25265-71-8	231	
2-(2-Butoxyethoxy)ethanol	112-34-5	225-234	
(2-Methoxymethylethoxy)propanol	34590-94-8	184-197	
1-Butoxypropan-2-ol	5131-66-8	171	
[(Butoxymethylethoxy)methylethoxy] propan-1-ol	55934-93-5	275	
1-Isopropyl-2,2-dimethyltrimethylene diisobutyrate	6846-50-0	281.5	

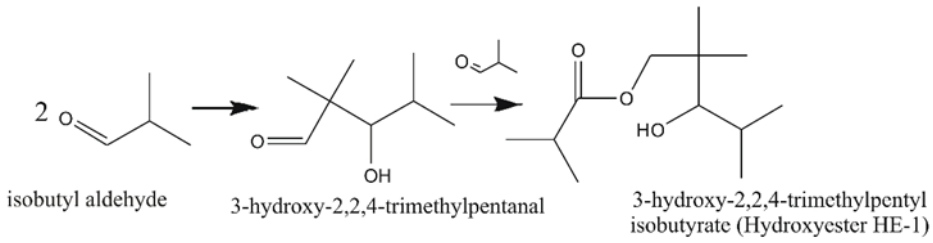


Fig. 1. General diagram of condensation of isobutanal to obtain hydroxyester HE-1.

2 Material and methods

2.1. Material

Hydroxyester HE-1 is obtained in a sequence of chemical reactions, where isobutyric aldehyde is the basic starting material in the process of aldol condensation with the subsequent Cannizaro and Tiszczenko reaction, as shown in the diagram in Fig. 1. HE-1 is hydrophobic solvent and its major application is a coalescent in water-based architectural paints.

The product has a boiling point of 255^{o}C and is not classified as a VOC [10, 11]. Since a HE-1 production plant of a capacity of more than 100 Mg per year is going to be launched, an assessment of the product in terms of its toxicological and ecotoxicological properties is required [12]. In this paper, the results of the human health risk tests of HE-1 are discussed. The toxicological tests were carried out in accordance with Good Laboratory Practices at the Institute of Industrial Organic Chemistry, Pszczyna, Poland.

2.2 Methods

The results of three selected toxicological tests of the hydroxyester HE1 are presented in this paper. They include, among other things: dermal irritation/corrosion test, eye irritation test, and bacterial reverse mutation test. Later in this paper, the hydroxyester HE-1 is compared, in terms of toxicological properties, with other advanced additives to paints and varnishes of which the CAS numbers, boiling temperatures and structural formulae are provided in Table 1.

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2.2.1 Acute dermal irritation/corrosion test in rabbit according to OECD Test Guideline No. 404 [13]

Grading the effects of acute dermal irritation/corrosion is an important step in the toxicity assessment of test materials, including the hydroxyester HE-1. The test results indicate the potential hazards of dermal exposure to the test material.

The objective of the test was to obtain information on the health-risk effect of potential dermal exposure to the test material. An initial test was carried out using one animal. A single 0.5 cm^3 dose of the test material was applied to a hairless skin site of one animal (rabbit 1) and secured with asuitable tape. The exposure time was 4 hours.

After the examination of the exposed skin, the test material was applied to the skin of two more animals (rabbits 2 and 3) for a period of 4 hours in order to confirm the presence or absence of irritant effect. The procedure was the same as in the application of the test material to the skin in rabbit 1.

For the duration of the experiment, all the test animals were subjected to general clinical observations for morbidity and mortality. Detailed clinical observations of the exposed skin in the test animals were conducted at 1, 24, 48 and 72 hours after exposure as well as at 7 and 14 days after exposure [14].

The results of the detailed clinical observation of the test animals were reported using the classification referred to in the OECD Test Guideline No. 404/ Method B.4. (Table 2).

Table 2. Grading of dermal reactions: erythema and eschar formation, oedema.

Erythema and eschar formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beef redness) to eschar formation preventing formation of erythema	4
Oedema	
No oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well defined by definite raising)	2
Moderate oedema (raised 1mm)	3
Severe oedema (raised more than 1 mm and extending beyond area of exposure)	4

According to [15], a substance or mixture applied to the skin in rabbit is classified as irritant after developing a clearly inflammatory condition which persists for at least 24 hrs after exposure.

The skin condition is clearly inflammatory if:
- mean grading for either the erythema and eschar formation or for the oedema is at least 2,
- both the erythema and eschar formation and the oedema formation were graded individually in two or three test animals as being equivalent to the mean score of at least 2 for the respective animals.

In both of the above cases, the mean values were calculated from all the reactions graded after 24, 48 and 72 hrs.

A clearly inflammatory condition of the skin will persist in at least two animals until the end of the observation. The following specific effects are taken into consideration: cell growth, desquamation, discoloration, cracking, eschar formation, hair loss [15].

2.2.2 Acute eye irritation/corrosion test in rabbit according to OECD Test Guideline No. 405 [16]

The objective of the acute eye irritation/corrosion test was to obtain information on the health-risk effect of potential exposure of the eyes to the hydroxyester HE-1.

According to the test procedure, 0.1 cm^3 of the hydroxyester HE-1 was placed in the conjunctival sac of one eye of each test animal whereas the other eye, which remained untreated, served as control. In order to confirm the presence or absence of irritation, the procedure was applied to three test animals. For the duration of the experiment, all the test animals were under general clinical observation, conducted daily for morbidity and mortality. Detailed clinical observations of the exposed eyes, regarding lesions of the cornea, iris, and conjunctiva were conducted at 1, 24, 48 and 72 hours after exposure.

For grading the acute eye irritation/corrosion effect, the following classification of lesions in the eye was applied (Table 3). The grading relates to the lesions in the cornea, iris and conjunctiva [17].

Table 3. Grading of lesions in the eye.

Cornea (opacity: degree of density)	
No ulceration or opacity	0
Scattered or diffuse areas of opacity, details of iris clearly visible	1
Easily discernible translucent area; details of iris slightly obscured;	2
Necrous area; no details of iris visible; size of pupil barely discernible	3
Opaque cornea; iris not discernible through the opacity	4
Iris	
Normal	0
Markedly deepened rugae, congestion, swelling, moderate circumcorneal hyperaemia; or injection; iris reactive to light	1
Hemorrhage, gross destruction, or no reaction to light	2
Conjunctivae – redness (refers to palpebral and bulbar conjunctivae; excluding cornea and iris)	
Normal	0
Some blood vessels hyperaemic (injected)	1
Diffuse, crimson color; individual vessels not easily discernible	2
Diffuse, beefy red	3
Conjunctiva – swelling (refers to lids and/or nictitating membranes)	
Normal	0
Some swelling above normal	1
Obvious swelling, with partial eversion of lids	2
Swelling, with lids about half closed	3
Swelling, with lids more than half closed	4

According to [15], a substance or preparation has irritant effect on the eye if, following test substance

application in the conjunctival sac of the test animal, it produces obvious lesions arising within 72 hours following application, which persist for at least 24 hours.

Ocular lesions are graded as obvious if mean scores correspond to any one of the following values:
- corneal opacity – grade 2 or higher than 2 but lower than 3;
- iridial lesion – grade 1 or higher than 1 but not higher than 1.5;
- circumcorneal hyperaemia – grade 2.5 or higher;
- conjunctival swelling – grade 2 or higher.

If three test animals were used in the test, the substance or preparation is considered to be irritant to the eye if ocular lesions in two or three test animals correspond to one of the grades referred to above, except for iridial lesion, where grade higher than 1 and lower than 2 applies, and for circumcorneal hyperaemia, where grade 2.5 or higher applies [15].

2.2.3 Bacterial reverse mutation test according to OECD Test Guideline No. 471 according to [18]

It was the objective of the test to determine the potential genotoxic effects produced by HE-1 in a standard Ames test – short-term mutagenicity test, recommended as a screen for genotoxic activity [19]. The bacterial reverse mutation test uses amino-acid requiring strains of *Salmonella typhimurium* to detect point mutations, which involve substitution, addition, or deletion of one or a few DNA base pairs. This test detects chemical substances producing mutations which revert mutations present in the test strains and restore the functional capability of the bacteria to synthesize an essential amino-acid. The revertant bacteria are detected by their ability to grow in the absence of the amino acid required by the parent test strain.

Two essential mutagenicity tests were used in the tests described in this paper: plate incorporation (without metabolic activation) and preincubation (with metabolic activation). In the plate incorporation method, components are mixed with an overlay agar and plated immediately onto minimal medium. In the preincubation method, the test mixture (bacteria + test substance) is incubated before being added to an overlay agar and plated onto minimal medium. In both techniques, after 2 or 3 days of incubation, revertant colonies are counted and compared with the number of spontaneous revertant colonies on solvent control plates. Mutagenicity of the test substance is shown in the growing count of the revertant colonies [20].

The test substance has a mutagenic effect if the concentration-related (over the range tested) and reproducible increase at one or more concentrations in the number of revertant colonies per plate with or without metabolic activation, $M_f > 2$ [20].
In order to determine mutagenicity, the result was also compared with the negative control, dimethyl sulfoxide (DMSO).

Initially, the dose range was selected by testing HE-1 at concentrations up to 5 mg/plate – the maximum test concentration according to the OECD Test Guideline No. 471. This preliminary experiment was performed to determine the HE-1 concentrations for the proper test. The following HE-1 test concentrations were used in the proper test: 0.0001, 0.001, 0.01, 0.1, 0.5, 1 mg HE-1/plate. The proper test assessed HE-1 for mutagenicity using the bacterial strains of *Salmonella typhimurium* TA 100, TA 98, TA 97, TA 1535 and TA 102. The tests were carried out in the absence of an external metabolic activation system – in the standard Ames plate test (without modification and with preincubation), and with a S9 fraction derived from the Sprague-Dawley rat and treated with Aroclor 1254 – in the standard plate test without modification. Three experiments without metabolic activation and two experiments with metabolic activation were performed.

3 Results and discussion

3.1 Acute dermal irritation/corrosion in rabbit according to OECD Test Guideline No. 404

Table 4 shows the results of the acute irritation/corrosion of the skin in two rabbits, graded from 0 to 4. The reaction of the test animals to the hydroxyester HE-1 was assessed by grading the skin reaction (Table 2). The acute irritation of the skin was assessed based on mean scores at 24, 48 and 72 hours.

At 1 hour after exposure, observation of the skin in the test site in rabbit 1 and rabbit 2 indicated a very slight erythema (barely perceptible). At 24 hours after exposure, the erythema was more intense and was graded as moderate to severe in the two rabbits. Moreover, a very slight (barely perceptible) oedema was observed in rabbit 1.

At 48 and 72 hours after exposure, the erythema in rabbit 1, which had lost some of its intensity by that time, was graded as well defined. Moreover, no oedema was found on the exposed skin in rabbit 1. During both observations, rabbit 2 continued to have a moderate to severe erythema and a very slight (barely perceptible) oedema. Moreover, epidermal desquamation was detected in that animal (Table 4). At 7 days after exposure, the skin of the two rabbits showed a very slight (barely perceptible) erythema and epidermal desquamation in the test site. The skin of rabbit 2 showed no oedema in the test site. 14 days after exposure, both rabbits (1 and 2) showed no pathological dermal changes in the test site (Table 4). At 24, 48 and 72 hours after exposure, mean value for erythema was 2.3 for rabbit 1 and 3.0 for rabbit 2. For oedema, mean values were 0.3 and 0.7, respectively (Table 4). Therefore, on the basis of the test results and according to [15], it is justified to say that the hydroxyester HE-1 causes skin irritation in rabbit.

3.2 Acute eye irritation/corrosion in rabbit according to OECD Test Guideline No. 405

The results of acute eye irritation/corrosion in rabbit are shown in Table 5. The effect of the hydroxyester HE-1 on the cornea, iris and conjunctiva was evaluated by scoring the degree of lesions in the eye in rabbit (Table 3). Following test substance application, lesions were observed in the cornea, iris and conjunctiva of the eye in rabbit.

Table 4. Grading of acute dermal irritation/corrosion in rabbit.

Rabbit No.	Reaction	Values at						Mean score at 24, 48 and 72 hours
		1 hour	24 hours	48 hours	72 hours	7 days	14 days	
1	Erythema	1	3	2	2	1 ED	0	2.3
	Oedema	0	1	0	0	0	0	0.3
2	Erythema	1	3	3 ED	3 ED	1 ED	0	3.0
	Oedema	0	0	1	1	0	0	0.7

ED – epidermal desquamation

Table 5. Grading of acute eye irritation/corrosion in rabbit.

Rabbit No.	Part of eye		Values at					Mean score at 24, 48 and 72 hours
			1 hour	24 hours	48 hours	72 hours	7 days	
1	Cornea		0	0	0	0	0	0
	Iris		0	1	0	0	0	0.3
	Conjunctiva	erythema	2	3	3	1	0	2.3
		swelling	1	1	1	0	0	0.7
2	Cornea		0	0	1	0	0	0.3
	Iris		0	1	0	0	0	0.3
	Conjunctiva	erythema	2	3	1	1	0	1.7
		swelling	2	2	0	0	0	0.7
3	Cornea		0	0	1	0	0	0.3
	Iris		0	1	0	0	0	0.3
	Conjunctiva	erythema	2	3	1	1	0	1.7
		swelling	1	1	0	0	0	0.3

At 1 hour following test substance application, no changes were observed in the cornea or iris of the eye in the three test rabbits. The conjunctiva of the eye in the three test rabbits showed a diffuse, crimson redness with individual vessels not easily discernible. Some swelling of the conjunctiva was observed in rabbits 1 and 3 and swelling with partial eversion of lids in rabbit 2.
The three test rabbits were observed to have hyperaemia with swelling of the nictitating membrane, circumcorneal injection and an exudate on the eye lids and lid hairs.

At 24 hours following test substance application in the three test rabbits, no changes were detected in the cornea, the iris was hyperaemic, and reaction of the pupil to light was correct. Diffuse beefy redness was observed in the conjunctiva in the three test rabbits. The test rabbits were observed to have the same conjunctival swelling as at 1 hour, that is, some swelling above normal in rabbits 1 and 3 and obvious swelling with partial eversion of the lids in rabbit 2. Moreover, each of the three test animals continued to have a hyperaemic and swollen nictitating membrane, and circumcorneal injection. An exudate on the eye lids and lid hairs was detected in the test rabbits 2 and 3 and a small amount of discharge in the test rabbit 1.

Based on the test results and [15], it is safe to say that the hydroxyester HE-1 is not an eye irritant/corrosive in rabbit.

3.3 Bacterial reverse mutation test according to OECD Test Guideline No. 471

For the bacteria *Salmonella typhimurium* TA 100, mean range of spontaneous mutations was 184-297 revertant colonies per plate. The ratio between the number of revertant colonies per plate and the number of revertant colonies per control plate was not observed to exceed 2. At a ratio of 1 mg/plate in the experiments with and without activation system, the number of revertant colonies was significantly reduced ($M_f < 0.8$).

For *Salmonella typhimurium* TA 1535, mean range of spontaneous mutations was 19-25 revertant colonies per plate. In one experiment without metabolic activation, a concentration of 1 mg/plate statistically significantly reduced the number of revertant colonies ($M_f < 0.6$). Another experiment, with metabolic activation, a concentration of 0.01 mg/plate produced a statistically significant decrease in the mutation frequency in comparison with the positive control, though without biological significance ($M_f = 0.78$).

For the bacteria *Salmonella typhimurium* TA 97, mean range of spontaneous mutations was 114-193 revertant colonies per plate. In one experiment without metabolic activation at a concentration of 1 mg/plate, a statistically significant increase in the mutation frequency in respect of the controls was detected, though without biological significance ($M_f = 0.66$).

In the experiment with metabolic activation at a maximum concentration of 5 mg/plate, the level of frequency of revertant colonies was reduced in comparison with the negative control (M_f = 0.35), and concentrations of 1 and 0.5 mg/plate significantly reduced the number of revertant colonies, though without biological significance.

For *Salmonella typhimurium* TA 102, mean range of spontaneous mutations was from 248 to 350 revertant colonies per plate. In the experiments without metabolic activation, the frequency range of revertant colonies was not higher in comparison with the negative control. In all the experiments, the number of revertant colonies was statistically significantly reduced at a concentration of 1 mg/plate (M_f < 0.77), comparably to 0.5 mg/plate in one experiment. It was observed that the toxicity of the concentrations 0.5 and 1 mg/plate was related to the duration of contact of the test substance with the test bacterial strain before plating it onto the Petri dishes. In the experiments with metabolic activation, HE-1 was not observed to increase the number of revertant colonies above the frequency range of spontaneous mutations.

For the bacteria *Salmonella typhimurium* TA 98, mean range of spontaneous mutations was 22-53 revertant colonies per plate. In the experiments without metabolic activation, the frequency range of revertant colonies was not higher in comparison with the negative control. In the experiments with metabolic activation, the mutagenic potential of HE-1 was not confirmed in the experiment with the fraction derived from the rat liver.

In the dose range from 0.0001 mg/plate to 1 mg/plate, HE-1 was not observed to increase the number of revertant colonies above the frequency range of spontaneous mutations. Figures 2 and 3 show the number of revertant colonies per plate for 5 different strains of *Salmonella typhimurium* with and without metabolic activation. All the results were compared with the negative control (DMSO). For the strain TA 102, at a HE-1 dose of 0.0001 mg/plate in the tests with and without metabolic activation, the number of revertant colonies was not determined.

From the data shown in Fig. 2 and Fig. 3, it was observed that the number of revertant colonies per plate was lower than that for the negative control regardless of the HE-1 dose in a majority of cases. For the bacterial strains TA 100 and TA 97 in the experiments without metabolic activation for a HE-1 dose of 0.001 mg/plate, the numbers of revertant colonies determined for the negative controls were exceeded, although not very highly: the percentages were 0.5 and 3%, respectively. A similar observation was made in the experiments with metabolic activation for a HE-1 dose of 0.0001 mg/plate and the strain TA 97 and for a HE-1 dose of 0.1 mg/plate and the strain TA 102, where the numbers of revertant colonies determined for the negative controls were slightly exceeded, by 1.4 and 1.8%, respectively.

The mutagenic effect test results indicate that HE-1 causes no statistically significant, dose-related increase in the number of revertant colonies and no statistically significant reproducible positive response to any of the test points. Based on the test results, the hydroxyester HE-1 was not found to produce a mutagenic effect in the test system.

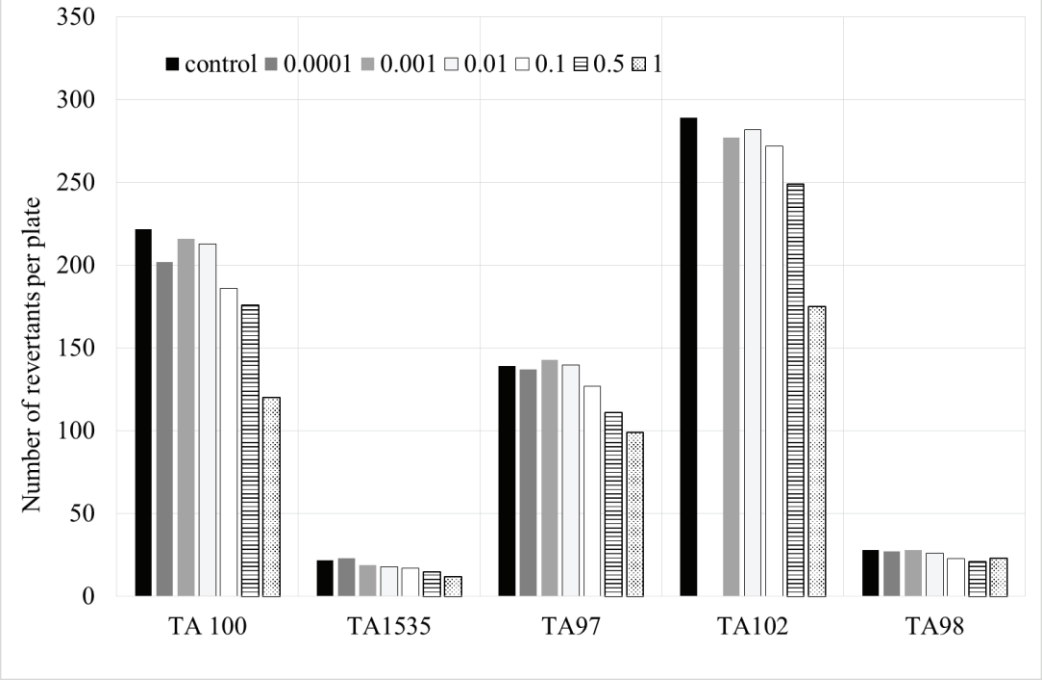

Fig. 2. Number of revertants per plate for various strains of *Salmonella typhimurium* without activation.

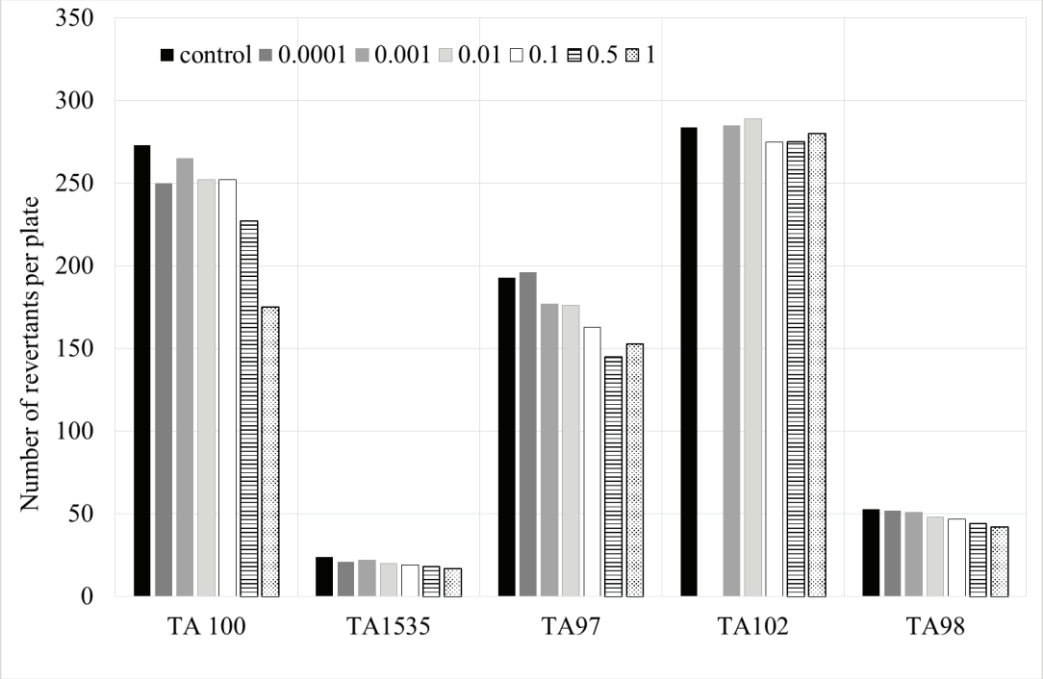

Fig. 3. Number of revertants per plate for various strains of *Salmonella typhimurium* with activation.

Table 6. Comparison of essential toxicological properties of advanced additives to paints and varnishes [6].

Compound	Acute skin irritation/corrosion	Acute eye irritation/corrosion	Mutagenic effect
57-55-6	no irritation/corrosion	no irritation/corrosion	no mutagenic effect
25265-71-8	no irritation/corrosion	no irritation/corrosion	no mutagenic effect
112-34-5	no irritation/corrosion	tests not according to OECD guidelines	no mutagenic effect
34590-94-8	no irritation/corrosion	no irritation/corrosion	no mutagenic effect **
5131-66-8	irritation	Irritation	no mutagenic effect
55934-93-5	no irritation/corrosion	no irritation/corrosion	no mutagenic effect
6846-50-0	no irritation/corrosion	no irritation/corrosion	no mutagenic effect **
HE-1*	irritation	no irritation/corrosion	no mutagenic effect

* - results of own tests
** - test on mammalian cells according to OECD 476

3.4 Comparison of toxicological properties of hydroxyester HE-1 and advanced additives to paints and varnishes

Table 6 shows the results of tests of the essential toxicological properties of HE-1 and advanced additives to paints and varnishes which are described in the literature and of which the names and structures were provided in Table 1. Information about the toxicological properties of advanced additives to paints and varnishes was collected from the ECHA website [9]. The following properties were compared: dermal and ocular irritation and mutagenic effect. For the compounds with the CAS numbers: 34590-94-8 and 6846-50-0, the mutation test was carried out using mammalian cells according to the OECD Test Guideline No. 476 [21]. Only three of the advanced additives to paints and varnishes shown in Table 6, namely those with the CAS numbers: 55934-93-5 and 6846-50-0 and HE-1, are not classified as VOC. When comparing their toxicological properties, it can be observed that HE-1 may have an irritant effect only when used at high concentrations. None of the compounds produces a mutagenic effect and none is an irritant. Although the other additives to paints and varnishes in Table 6 do not show any human health risk effect, their boiling temperatures are below 250^{o}C, therefore they are classified as VOC.

4 Conclusions

The results of toxicological tests are discussed on the basis of three selected examples: acute dermal irritation test, acute eye irritation/corrosion test, and mutagenic effect test.

For the acute dermal irritation/corrosion test in rabbit, mean score for erythema was 2.3 and 3.0, respectively, in rabbit 1 and rabbit 2. For oedema, mean score was 0.3 and 0.7, respectively, in rabbit 1 and rabbit 2. Therefore, based on the skin reaction grading, test results and [15], it is justified to say that the hydroxyester HE-1 is a skin irritant in rabbit at its high concentrations only.

In the acute eye irritation/corrosion test in rabbit, no lesions were detected in the cornea or iris in any of the test animals. Only erythema was observed in the conjunctiva in the three test rabbits, which manifested itself as injected blood vessels and congestion of the nictitating membrane. Based on the test results and [15], it is safe to say that the hydroxyester HE-1 does not produce eye irritation in rabbit.

In the mutagenic effect tests, HE-1 did not produce any statistically significant, dose related increase in the number of revertant colonies, and no statistically significant, reproducible positive response to any of the test points. The results of the mutagenic effect tests indicate that HE-1 is not mutagenic in the test system used.

When comparing the results of toxicological tests for the hydroxyester HE-1 and advanced additives to paints and varnishes, it is safe to say that HE-1 is an environmentally friendly alternative to those additives which either are classified as VOC or are involve human health risk.

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