Toxicity of Some Phenolic Derivatives—In Vitro Studies

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Cytotoxicity of 5 phenol derivatives (phenol, catechol, resorcinol, hydroquinone and phloroglucinol) was tested using a mouse 3T3 fibroblast cell line. Its relationships with structural and physicochemical properties were investigated.

Linear regression analysis and Pearson's correlation coefficient were used to characterise the relationship between cytotoxicity (expressed by IC_{50} values) and physicochemical parameters of compounds or their toxicity in vivo expressed by LD_{50} values. The studies showed that physicochemical properties of compounds seemed to have less influence on their cytotoxic potency than structural properties. Cytotoxicity of the compounds probably depends on the number of –OH groups and their location in the aromatic ring more than on physicochemical properties of compounds.

The best correlation was obtained for IC_{50} values and LD_{50} values determined following rabbit skin administration and experimental skin irritation score.

phenol   catechol   resorcinol   hydroquinone   phloroglucinol   cytotoxicity

1. INTRODUCTION

At present, in in vitro toxicology, the toxic effects of compounds are evaluated in stages. The first stage consists in determining general (basal) cytotoxicity [1, 2, 3, 4, 5, 6].

Basal cytotoxicity is defined as the interference of a chemical compound with structures and/or functions essential for survival and reproduction of almost any mammalian cell [4]. Non-differentiated proliferating cell lines are used for determining basal cytotoxicity. This stage allows priorities to be set for further investigations.

The aim of the studies of basal cytotoxicity conducted in recent years was, inter alia, to show general aspects of in vitro toxicology, therefore many various chemicals with different chemical structure and activity were studied.

A survey of the literature shows that only a limited number of papers have dealt with the problem; it is possible to draw conclusions about the cytotoxicity of very similar compounds on the basis of the methods used in basal cytotoxicity, which are considered to be standardised and validated enough. The possibility to assess the toxic potency of similar compounds would make it possible to predict possible adverse effects of untested chemicals if they were similar to the tested ones (for notification purposes).

The reliability of in vitro methods/tests is interpreted not only as reproducibility but also as concordance with in vivo data. For that reason many investigators commonly compare toxic values obtained from in vitro and in vivo experiments (IC_{50} and LD_{50} values). However in the whole organism toxicity of xenobiotics depends on different routes of administration, absorption, distribution and elimination pathways. The chemical structure and physicochemical properties of compounds decide about the aforementioned processes.

In the strategies of toxicity testing developed by the European Centre for Validation of Alternative

Methods (ECVAM), before performing basal cytotoxicity tests, it is recommended to conduct computer-based methods such as Quantitative Structure-Activity Relationships (QSARs) models [3, 7, 8].

A QSAR analysis relates the toxic activity of compounds to their structural properties. It is generally accepted that QSARs should be applied to a class of chemicals with a similar mode of action. Any compounds which do not have the same mechanism are likely to fit the correlation only poorly and to appear as "outliers" [7]. In the case of different compounds with a different mode of action, attempts to derive QSARs to predict their biological activity have not always been successful.

QSAR models are based on acute toxicity data usually derived from whole animal studies using LD_{50} or LC_{50} data. Recently there has been an increasing interest in the use of in vitro systems in conjunction with QSAR methods as a useful overall alternative to animal testing [7, 9, 10].

In this context, the present study was carried out. Its objective was to determine the relationship between the chemical structure and physicochemical properties of similar compounds and their biological activity expressed as basal cytotoxicity endpoints.

We tested the toxic effect of phenol, catechol (1,2-dihydroxybenzene), resorcinol (1,3-dihydroxybenzene), hydroquinone (1,4-dihydroxybenzene), and phloroglucinol (1,3,5-dihydroxytoluene). These compounds are widely used as components of drugs, pesticides and industrial chemicals (dyes, resins and adhesives). In addition, they are formed as biotransformation products in the metabolism of other occupational harmful agents (e.g., phenol, hydroquinone and catechol are metabolites of benzene; phloroglucinol is metabolite of phenol) [11].

The main effects of phenols are irritancy or corrosivity, depending upon the concentration (in the area of first contact), sometimes sensitization (hydroquinone, resorcinol). Hydroquinone and catechol have been shown to be carcinogenic and mutagenic agents in in vivo and in vitro studies [12].

Phenols are major components of cigarette tar and they are important factors in lung damage. They also have the propensity for radical formation [13]. Generally, most of the human population may be exposed to phenols. Therefore, assessment of their cytotoxic effects is of much significance. Cytotoxicity is regarded as an important step in developing such processes as irritation, inflammation, cell proliferation and hyperplasia, oxidative stress, damage and decreased organ function [14].

In the present study cytotoxicity of those compounds was evaluated on 3T3 fibroblasts after 3-hr exposure, using the MTT (3-(4,5 dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide) reduction assay, which assesses the metabolic activities of cells and the NRU (Neutral Red Uptake) assay measured cell membrane permeability.

2. MATERIALS AND METHODS

2.1. Chemicals

The following substances were used for cell cultures: Dulbecco's modified Eagle's medium (DMEM), foetal bovine serum (FBS) and Antibiotic-Antimycotic (penicillin G sodium, streptomycin sulphate, amphoterycin B as fungizone) from Gibco (Life Technologies Ltd., Paisley, UK). Sigma Chemical Co. (St Louis, MO, USA) supplied 0.25% Trypsin-0.02% EDTA solution and 0.4% trypan blue stain.

The following compounds were used in the test: MTT, NRU; Hank's Balanced Salt Solution, Dulbeco's Phosphate Buffered Saline, dimethylsulphoxide (DMSO) from Sigma; glacial acetic acid (100%) from Merck (Darmstadt, Germany); ethanol (96%) from Z.P.S. "Polmos" (Poland).

The test compounds, that is, phenol, 1,2-dihydroxybenzene (catechol), 1,3-dihydroxybenzene (resorcinol), 1,4-dihydroxybenzene (hydroquinone) and 1,3,5-dihydroxytoluene (phloroglucinol) were supplied by Merck

(Darmstadt, Germany) and were of higher than 98% purity.

2.2. Cell Culture

A Swiss albino mouse 3T3 fibroblast cell line was obtained from the German Collection of Microorganisms and Cell Cultures Dept. Human and Animal Cell Cultures (Braunschweig, Germany). They were cultured as a monolayer in a complete DMEM medium supplemented with 10% FBS and with antibiotic-antimycotic ($1 cm^3/100 cm^3$ medium) in sterile tissue culture flasks (Nunc, USA) and maintained at 37 ºC in a humidified atmosphere with 95% air: 5% CO_2 and pH 7.2–7.4. For subculturing, the cultures were trypsinized with 0.25% trypsin-0.02% EDTA. They were subcultured twice a week. Before starting the experiment, the cells were removed from the flask by trypsinisation and cell suspension was prepared. Cell number and cell viability were determined in a Bürker chamber by the trypan blue exclusion method [15]. Cells whose viability was over 95% were used in experiments. Experimental cell cultures were seeded at a density of 8×10^4 cells/well, with 100 μl medium in each well of the 96-well microplates (Nunc, USA) and cultured overnight to allow adherence and recovery from exposure to trypsin. After this period, non-attached cells were aspirated and test compounds were added.

Stock solutions were prepared in a medium immediately before each experiment. The medium with the test chemical was subsequently added to the cells after removing the growth medium, and incubated for 3 hrs at 37 ºC. A medium without a test compound was added to the control wells. Six different concentrations of each chemical were added to the cells. The concentrations were chosen on the basis of preliminary studies. After 3 hrs of exposure, viability of cells (reduction in the number of viable cells) was assessed with the NRU and MTT reduction assays.

2.3. Cell Viability Assays

2.3.1. NRU assay

The NRU assay is based on the uptake and lysosomal accumulation of the supravital dye, neutral red [16]. It was conducted according to INVITTOX Protocol No. 64 [17]. After removing the medium with tested chemicals, medium-containing neutral red dye (50 μg/cm³) was added to each well (100 μl). After incubation for 3 hrs, supernatants were removed and cells were fixed with 100 μl of 1% glacial acetic acid-50% ethanol-49% distillate water. The plates were left at room temperature for 10 min and shaken. Then absorption was measured using an ELISA microplates reader (at 540 nm against a 450 nm filter). The results were calculated for each concentration as a percentage of medium control and IC_{20}, IC_{50} and IC_{80} values (i.e., concentrations producing 20, 50 and 80 reduction of number viable cells) were established.

2.3.2. MTT assay

The MTT assay is based on the uptake and the reduction by mitochondrial succinate dehydrogenase of the soluble yellow MTT tetrazolium salt to an insoluble blue MTT formazan product [18, 19]. It was conducted according to INVITTOX Protocol No. 17 [20]. After removing the medium with tested chemicals, medium-containing MTT (5 mg/cm³ in Hank's buffered saline) was added to each well (100 μl). After incubation for 3 hrs, supernatants were removed and the violet formazan product obtained (by reduction of the MTT) was dissolved in 100 μl of pure DMSO. The plates were then shaken and absorption was measured using an ELISA microplates reader (at 570 nm against a 620 nm filter). The results were calculated for each concentration as a percentage of medium control and IC_{20}, IC_{50} and IC_{80} values were established.

2.4. Statistical Analysis

In both assays nine parallel runs were used for each concentration, and five independent experiments were carried out with each chemical. Viability of cells was calculated as the arithmetic mean percentages of control ± *SEM* (standard error of the mean). Non-linear estimation method (GraphPad Prism 3.03, San Diego, CA, USA, test version) was used to compute or extrapolate the concentration of the test agent needed to reduce viability of cell culture by 20, 50 and 80% (IC_{20}, IC_{50}, IC_{80} values). Significance of differences between IC_{20}, IC_{50} and IC_{80} values was determined with one-way analysis of variance (ANOVA) followed by Tukey's multiple range test for significant differences and the differences were considered significant if $p \le .05$.

The relationships between IC_{50} values and physicochemical parameters and LD_{50} values were evaluated using linear regression analysis and with Pearson's correlation coefficient.

3. RESULTS

3.1. Cytotoxicity of Compounds

The concentrations ($\mu g/cm^3$) that inhibited NRU and reduction of MTT salt are reported in Tables 1 and 2, respectively. A dose-dependent inhibition has been observed for all the compounds. Both assays allowed setting the concentration range in which the maximum (IC_{80}) and minimum (IC_{20}) toxic effect could be observed (Tables 1 and 2). The IC_{20}, IC_{50} and IC_{80} values calculated on the basis of MTT reduction assay were higher than calculated on NRU assay for all compounds.

The IC_{50} values were used as the main measure for comparing the cytotoxicities of phenol derivatives. Figure 1 presents a comparison of the IC_{50} values (expressed in $\mu mol/cm^3$) calculated for tested agents on the basis of NRU and MTT assays. The IC_{50} values for all compounds showed statistically significant differences at $p \le .05$, except for resorcinol and phloroglucinol in the MTT assay. The lowest IC_{50} values were

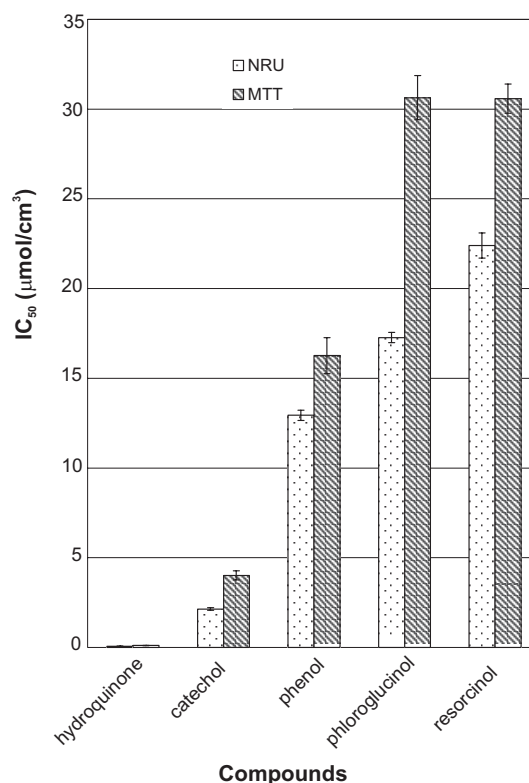observed for hydroquinone, whereas the highest for resorcinol.

Figure 1. Relative cytotoxicities of phenols to 3T3 cells. *Notes.* The cytotoxicities of hydroquinone, catechol, phenol, phloroglucinol and resorcinol were measured using NRU (Neutral Red Uptake) or MTT (3-(4,5 dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide) assay and are plotted in the order of the concentrations that inhibit cell growth by 50% compared with untreated controls (IC_{50} values). The results are *M ± SEM*.

3.2. Comparison of Cytotoxicity of Compounds With Their Physicochemical Properties

Table 3 presents a comparison of the IC_{50} values with some physicochemical properties of compounds performed on the basis of Pearson's correlation coefficient.

Generally correlation between in vitro cytotoxicity and most physicochemical properties of tested agents was poor. Only the relationship between IC_{50} values and solubility in water of compounds was good (Pearson's correlation coefficient was statistically significant $r = .71$ for NRU and $r = .73$ for MTT). When linear

TABLE 1. Cytotoxicity Effect of Tested Compounds on 3T3 Fibroblasts Expressed by IC_{20}, IC_{50} and IC_{80} Values for Neutral Red Uptake

Compounds	IC_{20} ($\mu g/cm^3$)				IC_{50} ($\mu g/cm^3$)				IC_{80} ($\mu g/cm^3$)			
	M	CI	SEM	Relative SD (%)	M	CI	SEM	Relative SD (%)	M	CI	SEM	Relative SD (%)
Hydroquinone (1,4-dihydroxybenzene)	4.4	0.77	0.4	19.9	7.0	0.98	0.5	15.9	11.2	1.54	0.79	15.7
Catechol (1,2-dihydroxybenzene)	180.2	8	3.9	4.9	236.0	17	8.6	8.1	310.4	39	19.9	14.3
Phenol	975.6	86	43.9	10.1	1217.4	53	27.0	5.0	1523.1	50	25.6	3.8
Phloroglucinol (1,3,5-dihydroxytoluene)	1276.3	97	49.7	8.7	2177.9	70	35.7	3.7	3732.1	257	131.1	7.9
Resorcinol (1,4-dihydroxybenzene)	1934.8	234	119.6	13.8	2466.2	150	76.7	7.0	3156.3	66	33.5	2.4

Notes. CI—confidence interval, SEM—standard error of the mean.

TABLE 2. Cytotoxicity Effect of Tested Compounds on 3T3 Fibroblasts Expressed by IC_{20}, IC_{50} and IC_{80} Values for MTT Reduction

Compounds	IC_{20} ($\mu g/cm^3$)				IC_{50} ($\mu g/cm^3$)				IC_{80} ($\mu g/cm^3$)			
	M	CI	SEM	Relative SD (%)	M	CI	SEM	Relative SD (%)	M	CI	SEM	Relative SD (%)
Hydroquinone (1,4-dihydroxybenzene)	7.0	1.51	0.77	24.5	12.0	1.64	0.84	15.5	20.9	2.37	1.21	12.9
Catechol (1,2-dihydroxybenzene)	205.2	47	24.1	26.3	441.9	55	28.0	14.2	967.2	96	49.0	11.3
Phenol	1176.6	203	103.6	19.7	1530.6	184	94.1	13.7	1998.9	177	90.2	10.1
Phloroglucinol (1,3,5-dihydroxytoluene)	1984.1	817	416.9	47.0	3863.4	303	154.6	9.0	8751.7	3204	1634.6	41.8
Resorcinol (1,4-dihydroxybenzene)	2851.1	214	108.9	8.5	3366.9	177	90.3	6.0	3979.5	151	77.1	4.3

Notes. CI—confidence interval, SEM—standard error of the mean.

Table 3. Comparison of in Vitro IC$_{50}$ Values and Physicochemical Properties for Phenol, Hydroquinone, Catechol, Resorcinol, and Phloroglucinol

Compounds	IC$_{50}$ (μmol/ cm^3) NRU	IC$_{50}$ (μmol/ cm^3) MTT	Log P	Mole-cular Volume (Å^3)	pKa	Melting Point (^{o}C)	Solubility in Water (g/dm^3)	Mole-cular Weight
Hydroquinone (1,4-dihydroxybenzene)	12.9	16.3	1.475	65	10.60	41	84	94.1
Catechol (1,2-dihydroxybenzene)	0.1	0.1	0.58	73	10.35	175	70	110.1
Phenol	2.1	4.0	0.806	73	9.85	106	450	110.1
Phloroglucinol (1,3,5-dihydroxytoluene)	22.4	30.6	0.58	73	9.81	113	1000	110.1
Resorcinol (1,4-dihydroxybenzene)	17.3	30.6	0.211	82	—	219	600	126.1
Pearson's correlation coefficient (NRU assay)			− .14	− .18	− .21	.03	.71	.16
Pearson's correlation coefficient (MTT assay)			− .30	− .37	− .28	.15	.73	.35

regression analysis was performed, the same results were observed. The highest dependence was found between IC$_{50}$ values and solubility in water (Figure 2). This analysis showed that there was no relationship between IC$_{50}$ values and such parameters as log P (logarithm of the octanol-water partition coefficient), pKa (dissociation constant), melting point, molecular weight and volume (R^2 = .02–.14; data not presented).
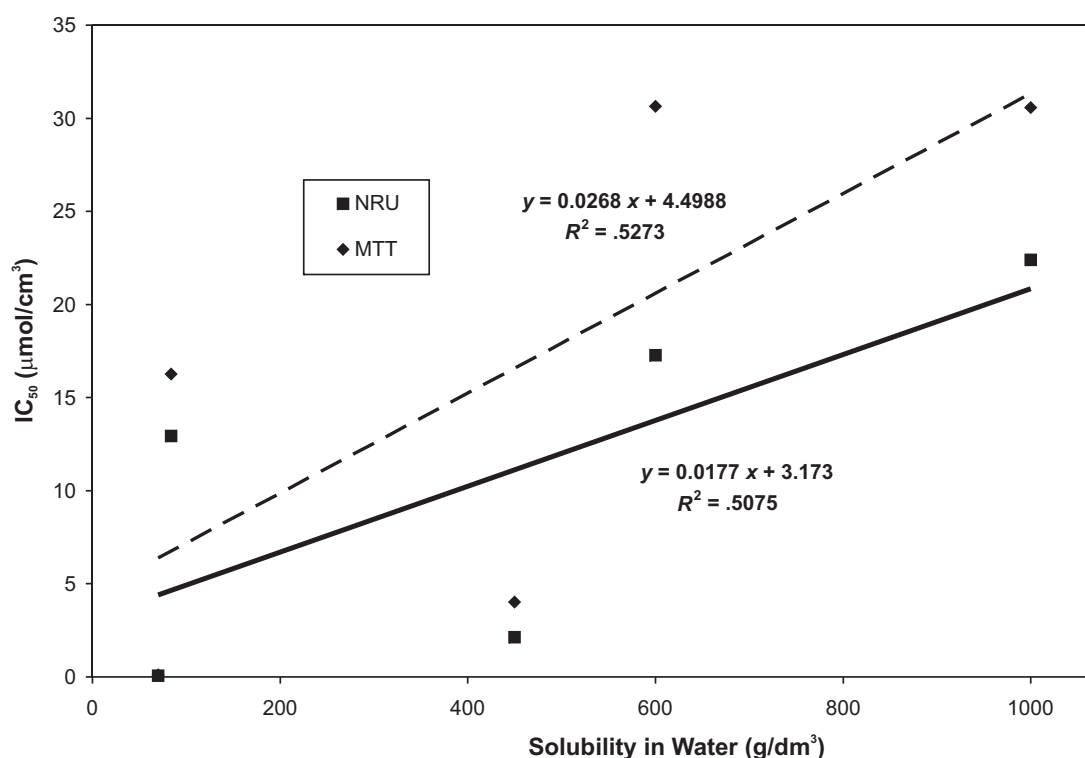

Figure 2. Relationship between cytotoxic concentrations (IC$_{50}$ values) of phenol, hydroquinone, catechol, resorcinol, and phloroglucinol and its solubility in water. *Notes.* NRU—Neutral Red Uptake, MTT—3-(4,5 dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide**.**

3.3. Comparison of In Vitro and In Vivo Toxicity of Compounds

Table 4 presents a comparison of IC_{50} values with in vivo acute toxicity expressed as LD_{50} values

(literature data) performed on the basis of Pearson's correlation coefficient. The highest values of Pearson's correlation coefficient were obtained for in vitro IC_{50} values and in vivo LD_{50}

TABLE 4. Comparison of in Vitro and in Vivo Acute Toxicities for Phenol, Hydroquinone, Catechol, Resorcinol, and Phloroglucinol

| Compounds	IC_{50} (μmol/ cm^3) NRU	IC_{50} (μmol/ cm^3) MTT	LD_{50} (Rat, Oral) (mg/kg)	LD_{50} (Mouse, Intraperito-neal/Intra-venous) (mg/kg)	LD_{50} (Rabbit, Skin) (mg/kg)	Experimen tal Skin Irritation Score
Hydroquinone (1,4-dihydroxybenzene)	12.9	16.3	396*	112	850	6
Catechol (1,2-dihydroxybenzene)	0.1	0.1	320	107	—	—
Phenol	2.1	4.0	322*	68	800	—
Phloroglucinol (1,3,5-dihydroxytoluene)	22.4	30.6	301	215	3360	1.125
Resorcinol (1,4-dihydroxybenzene)	17.3	30.6	4600*	4050	—	1.0
Pearson's correlation coefficient (NRU assay)			.37	.39	.86	− .83
Pearson's correlation coefficient (MTT assay)			.56	.58	.90	− 1.00

Notes. *—mean value.
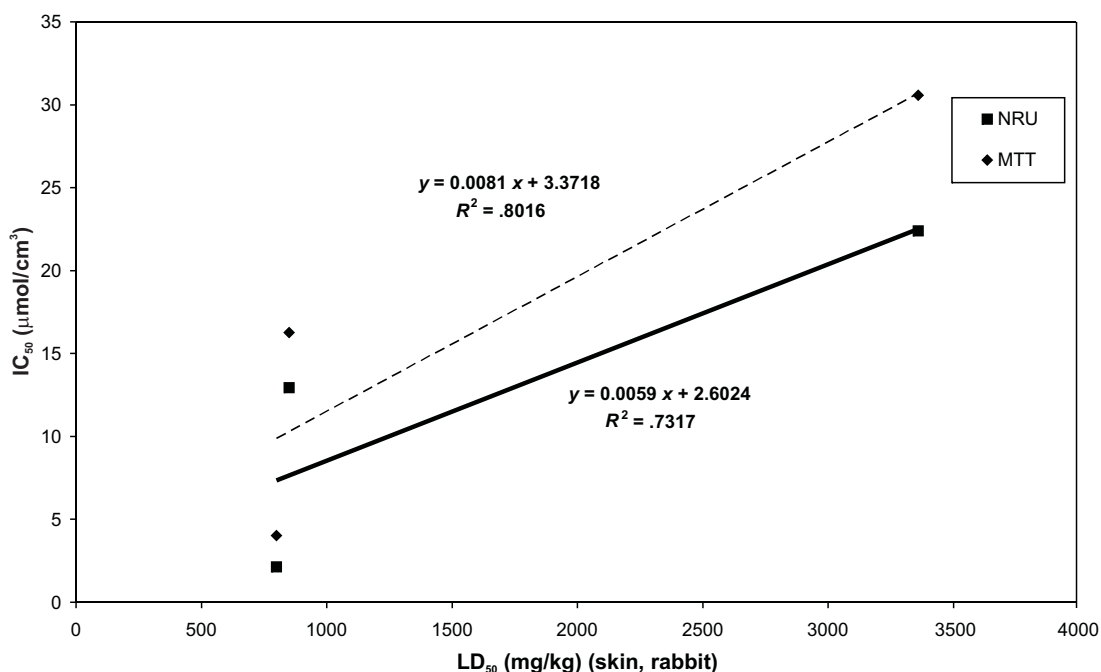
Figure 3. Relationship between cytotoxic concentrations for cultured cells (IC_{50} values) and LD_{50} (skin, rabbit) for phenol, hydroquinone, catechol, resorcinol, and phloroglucinol. *Notes.* NRU—Neutral Red Uptake, MTT—3-(4,5 dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide.

values determined following rabbit skin administration and experimental skin irritation score. Weaker correlation was obtained between IC_{50} values and LD_{50} values derived from oral rat and mouse intraperitoneal and intravenous data (Table 4). A similar effect was observed when linear regression analysis was performed (Figures 3–6).
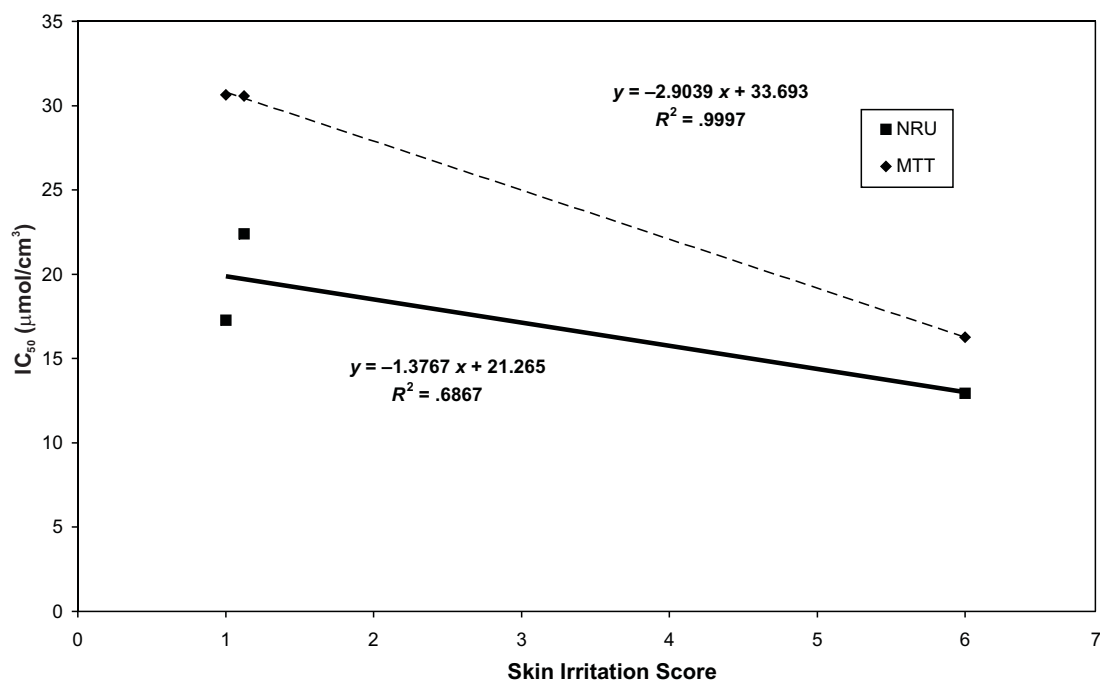

Figure 4. Relationship between cytotoxic concentrations for cultured cells (IC_{50} values) and skin irritation score for phenol, hydroquinone, catechol, resorcinol, and phloroglucinol. *Notes.* NRU—Neutral Red Uptake, MTT—3-(4,5 dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide.
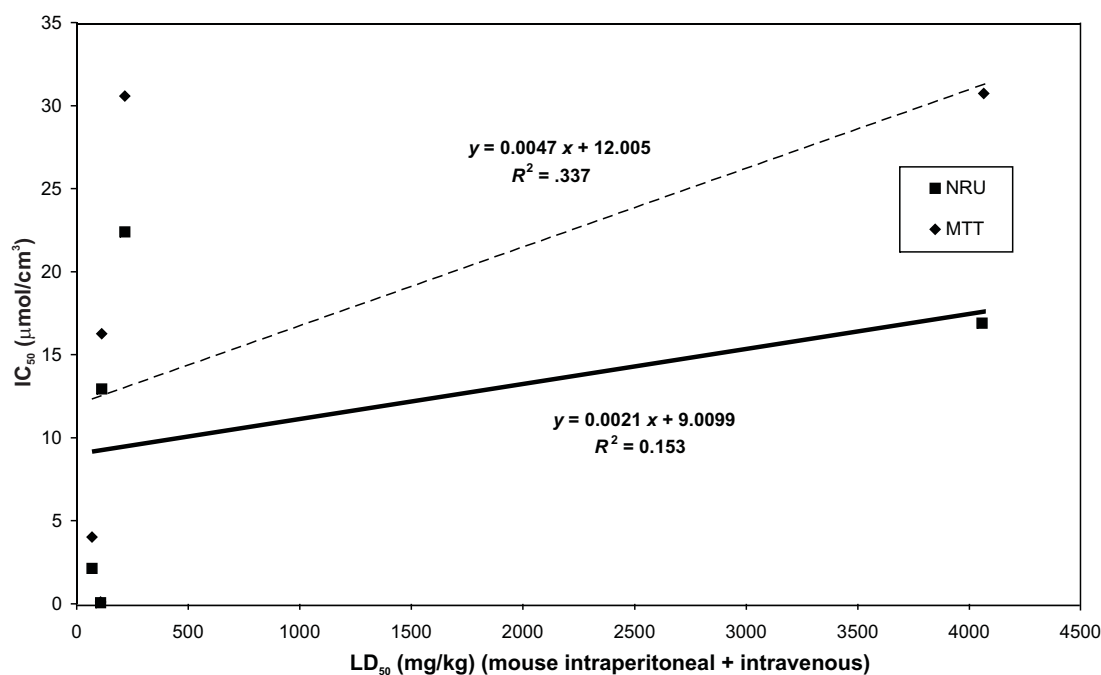

Figure 5. Relationship between cytotoxic concentrations for cultured cells (IC_{50} values) and LD_{50} (mouse, intraperitoneal and intravenous) for phenol, hydroquinone, catechol, resorcinol, and phloroglucinol. *Notes.* NRU—Neutral Red Uptake, MTT—3-(4,5 dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide.
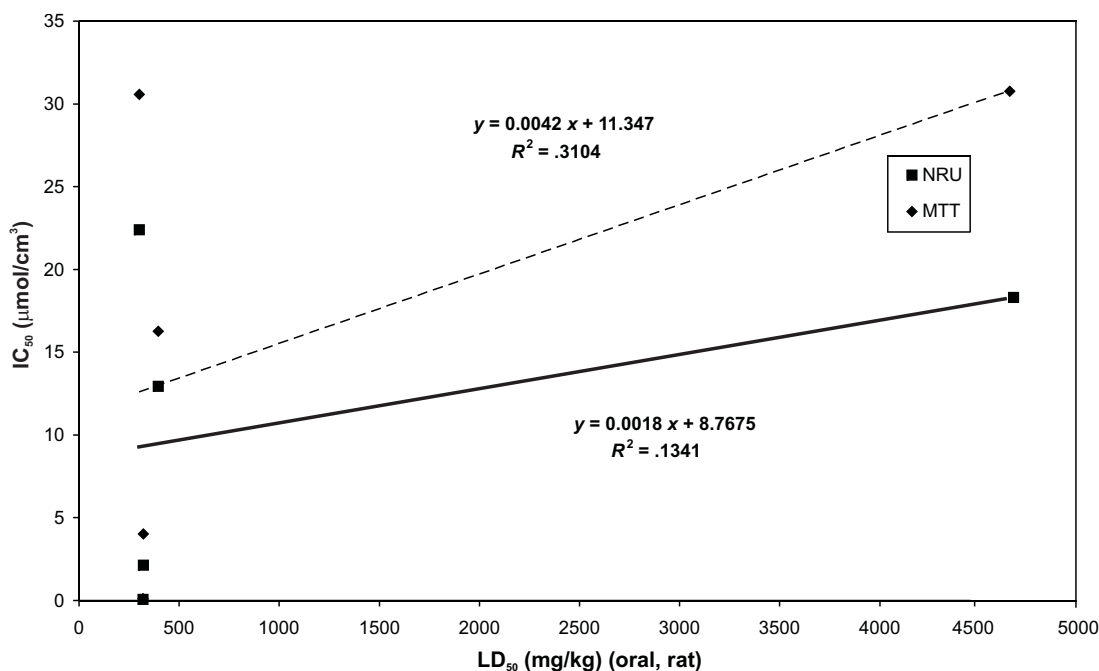
Figure 6. Relationship between cytotoxic concentrations for cultured cells (IC$_{50}$ values) and LD$_{50}$ (oral, rat) for phenol, hydroquinone, catechol, resorcinol, and phloroglucinol. *Notes.* NRU—Neutral Red Uptake, MTT—3-(4,5 dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide.

4. DISCUSSION

In accordance with ECVAM, two tests that measure different parameters of toxicity were chosen for assessing the cytotoxicity of phenols: the neutral red uptake assay estimate of the integrity of cell membrane (NRU test) and the reduction MTT assay estimate of the activity of mitochondria (MTT test) [2, 21, 22]. These methods have a very high level of standardization and validation [6, 23, 24]. At present they are the most frequently used ones in assessing the relative toxicity of chemicals in vitro [25, 26, 27, 28].

The cytotoxicity of phenol derivatives was investigated after 3-hr exposure of fibroblasts on tested compounds. For acute toxicity measurements, short exposure (3 hrs) is sufficient to start toxic action in the cells [27, 29]. After this time concentrations that induced 20, 50 and 80% (IC$_{20}$, IC$_{50}$ and IC$_{80}$ values) inhibition relative to controls were calculated.

The achieved results indicated that all of the tested compounds exhibited full cytotoxic effect. Both assays allowed setting the concentration range

in which the maximum (IC$_{80}$) and minimum (IC$_{20}$) toxic effect could be observed (Tables 1 and 2). It is worth noting that the NRU assay seemed to be more sensitive, that is, it was possible to observe cytotoxic effects at lower concentrations of tested compounds than in the MTT reduction assay. The NRU assay also proved to be more reliable—the lowest values of the SEM, confidence interval and relative standard deviation were obtained (Tables 1 and 2).

For comparison, cytotoxicity of phenol derivatives as the main measure the IC$_{50}$ values expressed in µmol/cm^3 was used. The IC$_{50}$ values are commonly used to express the cytotoxicity data in a manner that allows for the ranking of the test agents according to their potencies, and molarity seems a more appropriate unit for reporting data [5, 7, 30].

In the present experiments the tested agents had different cytotoxicity. Especially among the dihydroxybenzenes (hydroquinone, catechol, and resorcinol) very differentiated toxic potency was observed. The most toxic was hydroquinone, which contained a –OH substituent in the para

position of the phenol ring (p-isomer), the least toxic was resorcinol (m-isomer) (Figure 1). The toxic potency of the tested compounds was in the following order: hydroquinone, catechol, phenol, phloroglucinol and resorcinol.

One of the principal assumptions of the QSAR is that the behaviour of chemical compounds is dominated by their physicochemical properties. Traditional QSARs use experimentally derived descriptors such as, for example, log P, ionisation potentials [31], molecular volume or molecular weight [32]. These parameters are well known descriptors of skin permeability. Barrat et al. [7] used melting point as a parameter to describe solubility of substances, which depends on skin penetration. The results of QSAR analysis indicates that cellular toxicants are more lipophilic, less water-soluble, and exhibit higher mean molecular masses than non-toxicants [33]. Especially the logarithm of the octanol-water partition coefficient is used as a basal descriptor of the toxic action of compounds. A survey of the literature shows that toxicity of chemicals is primarily dependent on the hydrophobicity of the compounds and correlates with log P, even if the relationship can be improved by adding steric and electronic parameters [9, 34].

The regression analysis and Pearson's correlation coefficient indicated that cytotoxic endpoints were independent from log P. Especially the cytotoxicity of dihydroxybenzenes was more differentiated than predicted on the basis of log P considerations alone (Table 3). In the present experiment a poor relationship between IC_{50} values and pKa, melting point, molecular weight and volume was observed. This suggests that cytotoxicity of those compounds depends on other factors, probably on structural properties (on the number of –OH groups and their location in the phenolic ring, which have an effect on the steric and electronic parameters). It is difficult to interpret correctly the achieved results because the aforementioned observations are based on a small group of chemicals. Besides, the physicochemical properties of tested chemicals are similar which may make obtaining good

correlation difficult [7, 35]. Garg, Kurup, and Hansch [13] considered that maximum variance in activity with minimal collinearity in properties can be incorporated into the dataset in QSAR.

One of the most important problems in the evaluation of in vitro tests is a study of the relationships between the in vitro and in vivo data. Many studies have shown good correlation between in vitro cytotoxicity data and in vivo situations expressed as LD_{50} values determined following oral rat administration [2, 6, 36]. Fry et al. [37] have recommended the use of mouse intraperitoneal (i.p.) or intravenous (i.v.) LD_{50} values in preference to oral LD_{50} values. Other investigators also confirmed that the LD_{50} values obtained from intraperitoneal or intravenous routes are more appropriate for in vivo/in vitro comparison than are LD_{50} values determined following oral administration [6, 38].

In the presented data there was poor correlation between IC_{50} values and LD_{50} values derived from oral rat and mouse intraperitoneal and intravenous data. However, it can be seen from Table 4 that phenol and dihydroxybenzenes have very similar toxic potency expressed in vivo as LD_{50} (oral, rat and mouse i.p. and i.v.). The toxicity of these compounds assessed on the basis in vitro methods was more different.

The high value of Pearson's correlation coefficient and linear dependencies between LD_{50} (skin, rabbit) values or skin irritation score and IC_{50} values can indicate a local mechanism of toxic action of some phenol derivatives, but this would have to be confirmed on greater numbers of compounds.

5. CONCLUSIONS

1. The metods used to assess basal cytotoxicity (NRU and MTT) can be useful in determining the relationships between the structure and the activity of compounds.
2. The NRU assay was more sensitive and reliable than the MTT reduction assay.

3. The achieved results suggest that cytotoxic potency of the tested compounds depended rather on their structural parameters related to the reactivity of compounds (location of the OH group) than on their physicochemical parameters (especially the log *P* basal descriptor of the toxic action of most compounds).

4. The best correlation was obtained for IC_{50} values and LD_{50} values determined following rabbit skin administration and experimental skin irritation score.

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