

Toxicity of rubber chemicals in the chicken embryo

How to interpret results from animal tests

by Kari Hemminki, MD¹

HEMMINKI K. Toxicity of rubber chemicals in the chicken embryo: How to interpret results from animal tests. *Scand j work environ health* 9 (1983): suppl 2, 73–77. Results from 80 rubber chemicals in the chicken embryo test were reviewed and compared, when data were found, to results on teratogenicity in mammalian test systems. In addition other sources of literature were used to compare the chicken embryo test to the mammalian tests. Furthermore the results from animal models were analyzed in terms of their ability to predict teratogenicity in humans.

Key terms: embryotoxicity, predictive value of teratogenicity tests, teratogenicity testing.

A systematic study on the toxicity of about 80 rubber chemicals in the chicken embryo has been carried out during the past three years. The results have been published in eight original articles (8, 9, 10, 12, 13, 14, 15, 16) and also summarized recently (11). In the present article I want to discuss the meaning of the results obtained with the chick embryo test in relation to mammalian test systems and human teratogenic risks.

Results with the chick embryo

Korhonen and co-workers tested 80 rubber chemicals using the chick embryo model (8, 9, 10, 12, 13, 14, 15, 16). Three-day-old embryos were injected with 5 µl of acetone solution containing the test compound. The affected embryos were classified into the following categories: early deaths and nonmalformed and malformed survivors at day 14. The median effective dose (ED₅₀) was calculated for all the affected embryos (the aforementioned categories combined). The ED₅₀ values for all the

classes of rubber chemicals that could be tested are shown in table 1 according to their potency. Sixteen chemicals were negative in the test for reasons such as low solubility.

It is striking that large differences in the degree of embryotoxicity could be noted between classes of chemicals and even within individual classes. The dithiocarbamate derivatives were the most embryotoxic compounds, with ED₅₀ values of about 2–6 nmol/egg. On the other hand, for many groups of chemicals, the ED₅₀ values were in excess of 1 µmol/egg.

Extrapolation from chick tests

Although the chicken has been used for a long time in teratological testing, no exact information is available on what such tests actually measure. Opinions on the usefulness of the chicken embryo have been divided. A World Health Organization working group, giving no data to support its view, recommended that the chicken embryo should not be used in teratological testing (22). More recently the chicken embryo has gained increasing appreciation as an inexpensive and sensitive model that resembles mammalian morphological development and offers a kind of in vitro assay system (21).

¹ Department of Industrial Hygiene and Toxicology, Institute of Occupational Health, Helsinki, Finland.

Reprint requests to: Dr K Hemminki, Institute of Occupational Health, Haartmaninkatu 1, SF-00290 Helsinki 29, Finland.

Table 1. Ranking of classes of rubber chemicals according their toxicity in the chicken embryo.

Chemical	Median effective dose ($\mu\text{mol/egg}$)
Cadmium diethyldithiocarbamate	0.003
Zinc diethyldithiocarbamate	0.004
Zinc ethylphenyldithiocarbamate	0.004
Zinc dibutyldithiocarbamate	0.006
Copper dimethyldithiocarbamate	0.015
Tellurium diethyldithiocarbamate	0.026
Piperidinepentamethylenedithiocarbamate	0.160
Tetramethylthiuram disulfide	0.018
Tetramethylthiuram monosulfide	0.034
Tetraethylthiuram disulfide	0.091
Cyclohexylthiophthalimide	0.04
Phthalic anhydride	0.38
Butylbenzylphthalate	27
Dibutylphthalate	33
4,4'-Dithiodimorpholine	0.09
Acrolein	0.10
1,3-Butyleneglycoldimethacrylate	2.5
Tetrahydrofurfuryldimethacrylate	3.0
Trimethylolpropanetrimethacrylate	7.8
Methylmethacrylate	22
N-phenyl-N'-isopropyl-p-phenylenediamine	0.11
N,N,N-triethylethylenediamine	0.36
N,N'-dicyclohexyl-p-phenylenediamine	0.6
Triethylamine	0.9
Triethylenetetramine	1.1
N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine	1.5
Triethanolamine	2.6
N-phenyl-2-naphthylamine	3.0
Aniline	3.9
N-nitrosodiphenylamine	7.0
Cyclohexanone peroxide	0.13
Cumolhydroperoxide	0.16
Ethylmethylketoneperoxide	0.19
Dibenzoylperoxide	0.27
Acetylacetoneperoxide	0.34
Perbenzoic acid tert butylester	0.42
Dicumylperoxide	1.2
Dilauroylperoxide	1.8
Hydrogen peroxide	2.7
N,N'-di-o-tolylguanidine	0.17
N,N'-diphenylguanidine	0.20
1,3-Dibutylthiourea	0.28
1,3-Diphenylthiourea	0.41
Tetramethylthiourea	0.80
Trimethylthiourea	1.6
1,3-Diethylthiourea	3.1
1,3-Ethylenethiourea	4.5
N-t-butylbenzothiazylsulfenamide	0.30
N-oxydiethylene-2-benzothiazyl-sulfenamide	0.39
N-cyclohexyl-2-benzothiazyl-sulfenamide	1.0
2-Mercaptobenzothiazole	2.0
Resorcinol	2.4
Benzenesulfohydrazide	2.6
Tricresylphosphate	7.0
Synthetic aryl phosphate	9.5 mg
Highly aromatic oils	31 μg
Low aromatic, paraffin-based oils	87 μg
Naphthenic oils	480 μg
Polypropyleneglycoladipate	4.5 mg

Jelinek and co-workers have attempted a systematic evaluation of some of the parameters that the chicken embryo system measures; they compared the system to the mammalian test systems. Their test method "the chicken embryotoxicity screening test, phase I" measures the dose eliciting a significant decrease in the mean trunk length (6, 19). Results on the embryotoxicity of mycotoxins in the chicken embryo test were compared with the literature data on the toxicity of mycotoxins to mammalian cell cultures (fig 1). Generally a good correlation was found between embryoletality in the chick and toxicity in cell and tissue cultures (19). The same workers also extracted data from the literature on the mammalian LD₅₀ values for mycotoxins. A correlation was also found between embryotoxicity and the LD₅₀ values (fig 2). However, the variation in embryotoxicity was much larger (about 1,000-fold) as compared to the toxicity to mammals (about 40-fold).

In a separate work Jelinek & Rychter (6) compared the results from the chick system with those found for mammals in the literature (fig 3). Data on about 50 compounds were presented, and a relatively good correlation was reported. Yet again, like the data from fig 2, the effects on chicken embryos tended to span a wider dose scale than those on mammalian systems.

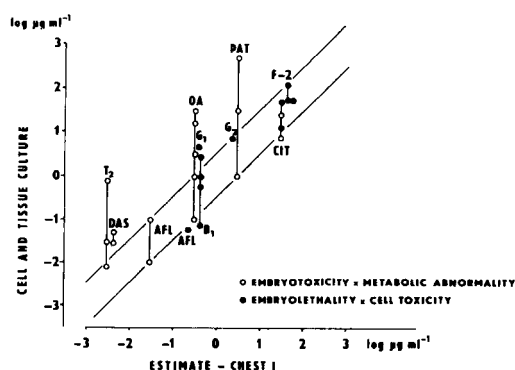


Fig 1. Correlation of results from the chick embryotoxicity screening test and cytotoxicity in cell and tissue cultures as elicited by the mycotoxins T-2 toxin (T₂) and diacetoxyscirpenol (DAS), the aflatoxins (AFL) B₁ (B₁), G₁ (G₁) and G₂ (G₂), ochratoxin A (OA), patulin (PAT), zearalenone (F-2), and citrinin (CIT). The figure has been taken from Vesley et al (19).

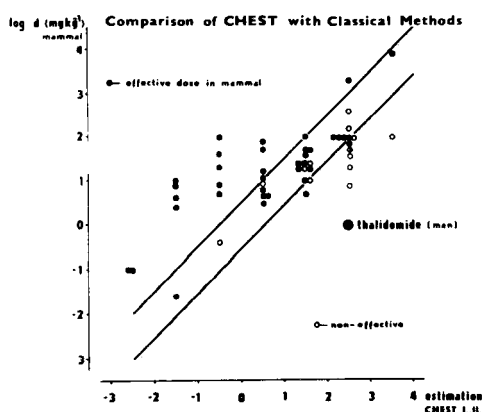


Fig 2. Correlation of the results with the chicken embryotoxicity screening test and median lethal doses (LD_{50}) of mycotoxins in vivo in mammals. The figure has been taken from Vesley et al (19). (T_2 = T-2 toxin, B_1 = aflatoxin B_1 , OA = ochratoxin A, PAT = patulin, CIT = citrinin, RB = rubratoxin B, ST = sterigmatocystin, PA = penicillic acid, p.o. = administered orally, i.p. = administered intraperitoneally, and i.v. = administered intravenously).

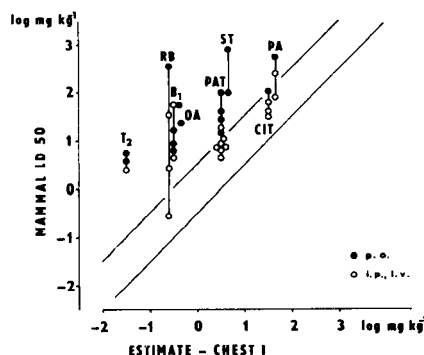


Fig 3. Comparison of the chicken embryotoxicity screening test with results of the mammalian test with 50 different chemicals, each being designated by a dot. The figure has been taken from Jelinek & Rychter (6).

Table 2. Comparison of results with chicken embryo and mammalian teratogenicity tests.

Substance	Median effective dose in chick (μ mol/egg)	Species	Dose (mg/kg)	Reference
Cadmium acetate	0.002	Hamster	2	Hemminki (5)
Mitomycin C	0.004	Mouse	5	Tanimura (17)
Cyclophosphamide	0.011	Monkey	10	Wilk et al (20)
		Rabbit, rat	2-50	Fritz & Hess (4)
				Chaube et al (2)
Tetraethylthiuram disulfide	0.018	Hamster	250	Hemminki (5)
Tetramethylthiuram disulfide	0.091	Hamster	125	Hemminki (5)
			(dimethyl sulfoxide)	
Copper (II) acetate, sulfate	0.096	Hamster	7 ^a	Hemminki (5)
Zinc acetate, chloride	1.0	Mouse	20 ^a	Chang et al (1)
Ethylenethiourea	4.5	Rat	20 ^a	Hemminki (5)
Methylmethacrylate	22	Rat	~300	Hemminki (5)
Sodium nitrite	22	Mouse	200	Teramoto et al (18)

^a Effects other than malformations reported.

The data obtained with the chicken system in our laboratory was compared with the literature results obtained with the same compounds in mammals (table 2). In the chick embryo test cadmium acetate, mitomycin C, cyclophosphamide, and tetramethylthiuram disulfide were potent embryotoxic agents (ED_{50} values between 0.002-0.018 μ mol/egg). The first three chemicals have also been shown to be potent teratogens in mammalian tests with

effective doses between 2 and 50 mg/kg. Tetramethylthiuram disulfide has proved to be a relatively weak teratogen. Tetraethylthiuram disulfide, copper (II) acetate, zinc acetate, and ethylenethiourea have been moderate in potency in the chick, as well as in mammalian systems. Copper and zinc salts caused toxic effects other than structural malformations in mammals. Methylmethacrylate and sodium nitrite were weak teratogens in the chick and

Table 3. Animal tests on chemicals with some evidence of teratogenicity in man after quantitated exposure.

	Effective dose in humans (mg/kg)	Animal data		Animal dose: human dose
		Species	Dose (mg/kg)	
Aminopterin ^a	0.02–0.04	Rat	0.07–0.2	~4
Androgens ^a	0.2	Rat	5–20	50
Cytostatic drugs ^a				
Busulfan	0.1	Rat	10–34	> 100
Chlorambucil	0.1	Rat	2–10	> 100
Cyclophosphamide	2	Rabbit	2–50	1–25
Diethylstilbestrol ^b	0.02–0.08	Monkey	0.2	2.5–10
Diphenylhydantoin ^a	6	Monkey	5–30	1–5
Ethanol ^c	1,800	Mice	5,000	3
Kanechlor ^c (poly- chlorinated biphenyls)	0.5	Monkey	0.1	0.2
Methotrexate ^b	0.04	Rat	0.2	5
Methylmercury ^c	1	Cat	0.25–4	0.2–4
Thalidomide ^a	1–10	Rabbit	0.5–50	0.5–5

^a From Hemminki (5).

^b Lowest effective dose (3).

^c Unpublished results of Hemminki & Vineis.

mammalian systems. However, the comparison clearly shows that the range of embryotoxicity is much larger in the chick system than in the mammalian systems.

In summary, the limited attempts to validate the chicken embryo in testing for embryotoxicity suggest that it is a sensitive model predicting toxicity and teratogenicity in mammalian systems.

Extrapolation from mammalian tests

Mammalian tests for teratogenicity are required by national agencies supervising food, drugs, pesticides, etc. Yet a systematic evaluation of the predictive value of the mammalian system has not been carried out. Some limited attempts in this direction have been reported (3, 5), and they will be discussed, while extensive surveys are still to be completed.

A feasible methodology by which the predictive value of animal tests can be evaluated is the analysis of the animal test results with compounds established or strongly suspected as human teratogens. Such a comparison has to be carried out in quantitative terms, as the dose is an important parameter in teratology. The type of comparison outlined earlier has been previously applied to carcinogens to validate the animal testing of carcinogens.

Table 3 contains data from previous reviews and some unpublished sources on compounds that are thought to cause

structural malformations or syndromes with typical structural or functional features. The effective human exposures were evaluated based on the original reports. For aminopterin, androgens, diphenylhydantoin, ethanol, methylmercury, Kanechlor 400 (containing polychlorinated biphenyls and polychlorinated dibenzofurans), and thalidomide the human dose that elicited frequent malformations was compared to results from animal tests. The data on cytostatic drugs came from case reports, and it is thus difficult to determine a true risk. For diethylstilbestrol and methotrexate the "lowest effective doses" (3) are given, and it is not clear to what part of the dose-response curve the data from experimental animals relates. As a further classification, the data are presented from sensitive animal species as milligrams per kilogram of body weight; no adjustment for different metabolic rates was attempted.

The comparison in table 3 suggests that the effective human and mammalian doses are relatively similar (animal dose/human dose = 0.2–10) for aminopterin, cyclophosphamide, diethylstilbestrol, diphenylhydantoin, ethanol, methotrexate, methylmercury, Kanechlor (polychlorinated biphenyls), and thalidomide. For androgens, busulfan, and chlorambucil man appeared to be much more sensitive. Some assumptions made in the comparisons are discussed in the original reviews, and they should be taken into

consideration before conclusions are drawn.

Conclusions

This limited survey on the validity of chicken and mammalian teratogenicity tests suggests that the chicken embryo test predicts cell toxicity and risk to the mammalian embryo. Furthermore the mammalian tests carried out with human teratogens seem to agree reasonably with the effective human doses. However, for predictive purposes, it may be important to apply results from a sensitive mammalian test system, because animal species and strains appear to vary greatly. Although comparisons have not been carried out between various species, monkey may be a relatively sensitive one, while the rodents rat and mouse may be relatively insensitive. One constitutive difference is the low capacity of rodent embryos to metabolize foreign compounds (5, 7). As a further point for extrapolation, it may not appear to be fruitful to compare individual types of lesions but, rather, to consider embryotoxicity in general. With such reservations, it appears that animal tests have some degree of predictive power to estimate potential hazards to the human embryo and fetus.

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