Bull. Environ. Contam. Toxicol. (1999) 63:660-664

Bulletin of Environmental Contamination and Toxicology

Sensitivity Evaluation of Bluebottle *(Calliphora vomitoria)* Larvae to Vapors of 10 Organic Solvents

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Received: 5 May 1998/Accepted: 30 July 1999

Organic solvents are commonly used in various industries because of their solubilizing, dispersant and diluent action in many processing stages. Many compounds are highly evaporable and it is worth remembering that the inhalation of certain vapors can seriously damage human health (Snyder and Andrews 1996). Bioassays can be very useful for the monitoring of such vapors and can integrate chemical analyses that, generally, give a limited evaluation to the very moment of the sampling, without any information about the interaction on organisms .

The purpose of the present study was to determine and to compare the effects of vapors from ten solvents (acetone, dimethysulfoxide, ethanol, methanol, hexane, heptane, octane, benzene, toluene, xylene) to two stages of development of Calliohora vomitoria ("flesh bluebottle") larvae, in order to evaluate the possibility of using such an organism as simple biological indicator in workplaces contaminated by the above mentioned vapors. The choice of Calliohora larvae is justified by its availability, paltry price, easy manipulation and by the peculiarities of its respiratory organs, dense of ducts (tracheae and tracheoles) which constitute a large surface for gas-exchanges .

MATERIALS AND METHODS

In the following tests apodal larvae of Calliphora vomitoria, available all year round at fishing-shops and well-known as " tallow maggots", were used. The stage of larval development examined showed the following average parameters: length 14.8 mm, weight 94.7 mg , volume 96 mm^3, diameter 3.25 mm, about 18 days old. The more vital organisms were selected on the basis of their capacity to climb over a 500 ml-beaker wall, with vertical run of about 12.5 cm. Groups of 30 larvae, obtained after selection, were distributed in fit airtight glass vessels of 5 dm^3 in volume, for each tested dose and for controls. Different aliquots of the ten solvents, analysis grade, were put in the

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containers, in the following doses (in µL/dms, at 20 °C): acetone, ethanol 96%, methanol, n-hexane, n-heptane, iso-octane, benzene, toluene, xylene: 1, 2, 4, 8, 16, 32, 64, 128 and for dimethylsulfoxide (DMSO) also 256, corresponding to the following concentrations (in g/m^3): acetone and methanol:0.79, 1.58, 3.16, 6.32, 12.64, 25.28, 50.56, 101.12; ethanol 96%: 0.81, 1.62, 3.24, 6.48, 12.96, 25.92, 51.84, 103. 68; DMSO: 1.1, 2.2, 4.4, 8.8, 17.6, 35.2, 70.4, 140.8, 281.6; n-hexane: 0.66, 1.32, 2.64, 5.28, 10.56, 21.12, 42.24, 84.48; n-heptane: 0.68, 1.36, 2.72, 5.44, 10.88, 21.76, 43.52, 87.04; iso-octane: 0.69, 1.38, 2.76, 5.52, 11.04, 22.08, 44.16, 88.32; benzene: 0.88, 1.76, 3.52, 7.04, 14.08, 28.16, 56.32, 112.64; toluene: 0.87, 1.74, 3.48, 6.96, 13.92, 27.84, 55.68, 111.36; xylene: 0.86, 1.72, 3.44, 6.88, 13.76, 27.52, 55.04, 110.08. In order to aid the evaporation of the solvents , which are liquid at room temperature, solvents were placed on a filter paper of about 40 cm^2 (3 x 13.3 cm) hanging by a thin thread at the interior tops of the different containers, so as to avoid the direct contact with the organisms and to allow their exposure to the vapors stratified underneath. A separate filter was moistened with 1 ml of distilled water and mounted into each container to provide the adequate internal humidity for the larvae. Every 5 days the above mentioned vessels were opened, both filters (the one of the solvents and the other one used for humidification) were removed and the inside gases carefully blown away by an air flux such as to avoid damages to the larvae or pupae, in a set time not less than 20 minutes, sufficient to eliminate the compounds used for the test. After this treatment, the substances under examination were put on a new filter, respecting the previously fixed dosage, and the humidity also was restored. The substitution, carried out in order to allow the constancy of vapor concentrations and the renewal of the consumed oxygen, was effected at the above mentioned frequency till the first emergences. Every day the organisms were checked, through the glass, in order to notice any changes inside each container. On average, the pupation was completed within 5-8 days, while the adults developed in 20-30 days. The hatchings were counted only when the flies had completely freed themselves from the puparium and were able to move The reference time was that of the control, with the achievement of the 100% of the events (pupae and/or emergences). The containers were kept, for the whole period of bioassay, at an average temperature of 20° C (±1°C), in a thermostatic room. For every tested dose three replicates have been carried out: the LC_{50} was calculated using the Spearman-Karber method (Finney 1952) since the doses were distributed on a geometrical scale and were able to determine effects variable from 0 % to 100 % (with the exception of DMSO in the larva-pupa stage). The 95% confidence limits have been obtained calculating the standard error of

LC_{50} as predicted by the above method. Three groups of compounds with similar chemical characteristics have been considered, in order to compare the effect of the various solvents. They are the following: group 1 (two alcohols and one ketone, containing one atom of oxygen); group 2 (three alkanes); group 3 (three aromatics). DMSO has not been considered because it was impossible to calculate LC_{50} in the stage larva-pupa. The significant differences between the various solvents, in the three groups, were determined by an analysis of variance procedure (ANOVA), followed by post hoc contrast with Student-Neuman-Keuls test, while the comparison of the two stages (larva-pupa: L-P and pupa-hatching: P-H) of the same compound was estimated according to the Student's t test for paired data (Glantz 1981).

RESULTS AND DISCUSSION

The different LC_{50}s expressed in g/m^3, and the relative confidence limits obtained with the various solvents are reported in Table 1. All the control tests were 100% successful in the pupation and hatching. The results show a dose-effect response of Calliphora vomitoria larvae to the vapors of the solvents used in the test, as well as a significantly different sensibility (Table 2) between the first stage of development (L-P) and the second one (P-H), which shows smaller LC_{50} values in all tested compounds . Such an effect may be due to the lower duration and biological complexity of the first stage (Chinery 1985). Comparing the same stages (L-P to L-P and P-H to P-H) to the use of different substances in the three groups considered, 1 (methanol, ethanol, acetone); 2 (hexane, heptane, octane); 3 (benzene, toluene, xylene), significative differences have been observed only in the first two groups, while the third one has not shown any significative difference: this means that the tested aromatic compounds have proved a similar toxic effect (Table 2). LC_{50} values reported in Table 1 show the following decreasing degree of toxicity: benzene, xylene, toluene, methanol, acetone, ethanol, octane, heptane, hexane, DMSO. The DMSO toxicity is the lowest for both development stages and, in fact, it was not possible to determine the LC_{50} of the first one (L-P) since the highest dose used (281.6 g/m^3) developed only 6.7 % of mortality at the saturation of vapors and, for this reason, it was impossible to compare them to the others.

Since the results show different sensibility of Calliphora vomitoria to the solvents tested, it is worth continuing an investigation of this potential test system in order to detect gaseous polluting substances in confined

spaces. This can be obtained by simple biological indicators such as, for instance, dipterous larvae. The results show also that the LC_{50} values of the tested compounds were much higher than the relative TLVs-TWA (threshold limit values-time weighted average) fixed by the ACGIH (1989) in the workplaces. Therefore the possible mortality, in such environments, of larvae during one of two stages of development indicated clearly that the vapors of solvents had exceeded safety limits for human health.

Table 1. LC_{50} values, relative confidence limits at 95% and TLVs-TWA (expressed in g/m^3) of vapors of the solvents used, on the two development stages of Calliphora vomitoria .

		LC_{50}	confidence limits		TLV-TWA
benzene	L-P	4.138	3.588	4.773	0.032
"	P-H	1.886	1.628	2.185	
xylene	L-P	4.539	4.054	5.083	0.436
"	P-H	1.931	1.650	2.260	
toluene	L-P	5.154	4.333	6.131	0.377
"	P-H	1.999	1.718	2.326	
methanol	L-P	10.028	8.830	11.389	0.262
"	P-H	3.465	2.980	4.029	
acetone	L-P	13.241	11.356	15.439	1.780
"	P-H	5.252	4.457	6.189	
ethanol 96%	L-P	17.101	15.033	19.453	1.880
"	P-H	9.829	8.479	11.393	
iso-octane	L-P	17.935	15.216	21.138	1.400
"	P-H	9.836	8.639	11.198	
n-heptane	L-P	24.425	21.262	28.058	1.640
"	P-H	14.692	12.423	17.375	
n-hexane	L-P	37.632	33.412	42.384	0.176
"	P-H	24.828	22.207	27.758	
DMSO	L-P	N.D.	N.D.	N.D.	N.R.
"	P-H	61.329	52.325	71.883	

L-P: larva-pupa P-H: pupa-hatching

N.D.: not determinable N.R.: not reported

Table 2. Comparison of vapor toxicity of the solvents tested, on the two development stages of Calliphora vomitoria

GROUP 1		ethanol 96%		methanol		acetone	
		L-P	P-H	L-P	P-H	L-P	P-H
ethanol 96%	L-P	--------	S(**)a	S(**)	--------	S(**)	--------
"	P-H	S(**)a	--------	--------	S(**)	--------	S(**)
methanol	L-P	S(**)	--------	--------	S(**)a	S(**)	--------
"	P-H	--------	S(**)	S(**)a	--------	--------	S(**)
acetone	L-P	S(**)	--------	S(**)	--------	--------	S(**)a
"	P-H	--------	S(**)	--------	S(**)	S(**)a	--------
GROUP 2		n-hexane		n-heptane		iso-octane	
		L-P	P-H	L-P	P-H	L-P	P-H
n-hexane	L-P	--------	S(**)a	S(**)	--------	S(**)	--------
"	P-H	S(**)a	--------	--------	S(**)	--------	S(**)
n-heptane	L-P	S(**)	--------	--------	S(**)a	S(**)	--------
"	P-H	--------	S(**)	S(**)a	--------	--------	S(**)
iso-octane	L-P	S(**)	--------	S(**)	--------	--------	S(**)a
"	P-H	--------	S(**)	--------	S(**)	S(**)a	--------
GROUP 3		benzene		toluene		xylene	
		L-P	P-H	L-P	P-H	L-P	P-H
benzene	L-P	--------	S(**)a	NS	--------	NS	--------
"	P-H	S(**)a	--------	--------	NS	--------	NS
toluene	L-P	NS	--------	--------	S(**)a	NS	--------
"	P-H	--------	NS	S(**)a	--------	--------	NS
xylene	L-P	NS	--------	NS	--------	--------	S(**)a
"	P-H	--------	NS	--------	NS	S(**)a	--------
L-P: larva-pupa			P-H: pupa-hatching			a: "t" test	
NS: not significative			S(**): p< 0.01				

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