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DOSE-RESPONSE RELATIONSHIPS IN HUMAN EXPERIMENTAL EXPOSURE TO SOLVENTS

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□ Previous studies carried out in the field of experimental toxicology have shown evidence of biphasic dose-response relationships for different experimental models, endpoints and chemicals tested. As these studies excluded humans as the experimental model, we have examined the literature of the last three decades in order to verify data concerning human experimental exposure with the aim of highlighting possible biphasic dose-response relationships. The substances used for experimental exposures included hydrocarbons, esters, alcohols, ketones, ethers, glycoethers, halogenated hydrocarbons, and carbon sulphide; the absorption route was inhalation. We did not detect any biphasic dose-response relationship and, in the studies reviewed, our examination revealed major methodological limitations that prevented us making a more detailed examination of experimental data. We concluded that the experimental data available did not allow us to support evidence of biphasic dose-response relationships in human experimental exposure to the above-mentioned chemical substances.

Keywords: solvents, humans, exposure, dose-response relationship

INTRODUCTION

Hormesis is a phenomenon characterized by a dose-response relationship with stimulation at low doses and inhibition at high doses. This relationship is therefore biphasic, i.e. U- or inverted U-shaped (Calabrese and Baldwin 2003).

A number of experimental studies have provided evidence of hormetic dose-response relationships that until recently were the subject of a certain scepticism on the part of the scientific community. These findings have resulted in hormesis being recognised as a general principle of Toxicology (Eaton and Klaassen 2001; Beck *et al.* 2001). In a careful review of thousands of articles published in the toxicological and pharmacological literature in the latter years of the twentieth century, Calabrese and Balwin (2001a; 2001b) made a decisive contribution to proving the existence of a large number of hormetic dose-response rela-

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tionships. Their report demonstrated that hormesis is a phenomenon that occurs in a wide range of biological models, endpoints, and substances from different chemical classes. The review by Calabrese and Baldwin (2001a; 2001b) to verify the hormetic phenomenon did not include "epidemiological studies, and field studies". Moreover, there are no studies in the literature aimed at evaluating biphasic dose-response relationships either in occupationally exposed subjects or in volunteers experimentally exposed to chemicals.

The aim of this study was to examine the literature on human experimental exposure by inhalation to several volatile organic substances widely used in industry in order to verify the existence of biphasic dose-response relationships.

MATERIALS AND METHODS

The chemicals studied in order to reveal eventual biphasic dose-response relationships were as follows:

- Hydrocarbons: *n*-hexane, styrene, xylene, terpens, toluene, trimethylbenzenes and cyclohexane;
- Esters: *n*-butyl acetate, methyl formate, ethylene glycol monoethyl ether acetate;
- Alcohols: isopropanol, 1-octanol, 2-butoxyethanol and cyclohexanol;
- Ketones: acetone, methyl *n*-butyl ketone, methyl ethyl ketone, methyl isobutyl ketone;
- Ethers: methyl tertiary butyl ether;
- Glycoethers: ethylene glycol monoethyl ether, 1 metoxy-2-ethanol, 1 metoxy-2-propanol;
- Halogenated hydrocarbons: 1,1,1-trichloroethane, 1,1,1,2-tetrafluoroethane, trichlorofluoromethane, 1,1,2-trichloro-1,2,2-trifluoroethane, dichlorodifluoromethane, methylene chloride, methyl chloride, tetrachloroethylene, trichloroethene, 1,1,1,2-tetrafluoroethane and 1,1,1,2,3,3,3-heptafluoropropane;
- Carbon sulphide.

Using the chemical name of each of the aforementioned substances as the search keyword, we carried out a Medline data search for the years 1965 – 2003. To reduce the number of papers found on Medline we then repeated the search using the chemical name coupled with the following additional key words: occupational, exposure, inhalation or volunteer. Furthermore a number of articles were obtained by cross-referencing journal citations.

From this group of papers we then excluded those referring to co-exposures, field studies and articles not written in English. The remainder, which we called "selected papers", refer only to controlled human

laboratory exposures, and were subsequently screened to eliminate those with less than three exposure doses, including an eventual control dose. Finally, with the exclusion of papers in which exposures did not remain constant throughout, we obtained the "admitted papers" used in this study. The dose-response relationships reported in the admitted papers included physiological or clinical parameters as a response.

STATISTICAL EVALUATION

For each article admitted, statistics, size and modality of selection of sample have been evaluated.

RESULTS

The admitted papers are reported in Table 1 according to the substance, its CAS number, exposure level (mg/m^3 or ppm) and duration (min), and exercise (W).

TABLE 1: Summary of the data on the admitted papers.

Chemical class, Substance, CAS no.	Doses and exposure duration	Exercise	Number of subjects	References
Hydrocarbons				
Styrene 100-42-5	51.4 ppm (60 min)	NS	3	Stewart et al. (1968)
	202.9 ppm (60 min)		3	
	368.4 ppm (60 min)		5	
	26 ppm (120 min)	50 W	2	Löf and Johanson
	77 ppm (120 min)		2	(1993)
	201 ppm (120 min)		2	
	386 ppm (120 min		2	
d-Limonene 5989-27-5	10 mg/m^3 (120 min)	50 W	8	Falk-Filipsson et al.
	225 mg/m^3 (120 min)		8	(1993)
	450 mg/m^3 (120 min)		8	
α-Pinene 80-56-8	10 mg/m^3 (120 min)	50 W	8	Falk et al. (1990)
	225 mg/m^3 (120 min)		8	
	450 mg/m^3 (120 min)		8	
3-Carene 13466-78-9	10 mg/m^3 (120 min)	50 W	8	Falk et al. (1991)
	225 mg/m^3 (120 min)		8	
	450 mg/m^3 (120 min)		8	
Toluene 108-88-3	50 ppm (240 min)	At rest	6	Veulemans and
	100 ppm (240 min)		6	Masschelein (1978a)
	150 ppm (240 min)		6	
	50 ppm (240 min)	At rest	6	Veulemans and
	100 ppm (240 min)		6	Masschelein (1978b)
	150 ppm (240 min)		6	
	50 ppm (240 min)	NS	6	Veulemans and
	100 ppm (240 min)		6	Masschelein (1979)
	150 ppm (240 min)		6	
	0 ppm (120 min)	NS	16	Andersen et al.
	10 ppm (120 min)		16	(1983)
	40 ppm (120 min)		16	
	100 ppm (120 min)		16	

Table 1 continued on next page

TABLE 1: Continued.

Chemical class, Substance, CAS no.	Doses and exposure duration	Exercise	Number of subjects	References
Hydrocarbons				
Toluene 108-88-3	45 ppm (480 min)	NS	4	Kostrzewski and
	81 ppm (480 min)		4	Piotrowski (1991)
	131 ppm (480 min)		4	
1,3,5-trimethylbenzene	10 mg/m^3 (480 min)	NS	5	Kostrewski and
108-67-8	25 mg/m^3 (480 min)		5	Wiaderma-Brycht
	50 mg/m^3 (480 min)		5	(1995)
	100 mg/m^3 (480 min)		5	
	150 mg/m^3 (480 min)		5	
	10.2 mg/m^3 (480 min)	NS	5	Kostrewski et al.
	24.4 mg/m^3 (480 min)		5	(1997)
	50.8 mg/m^3 (480 min)		5	
	97.9 mg/m^3 (480 min)		5	
	151.1 mg/m^3 (480 min)		5	
1,2,4-trimethylbenzene	9.6 mg/m^3 (480 min)	NS	5	Kostrewski et al.
95-63-6	24.8 mg/m^3 (480 min)		5	(1997)
	49.2 mg/m^3 (480 min)		5	
	106.8 mg/m^3 (480 min)		5	
	154.0 mg/m^3 (480 min)		5	
1,2,3-trimethylbenzene	5.0 mg/m^3 (480 min)	NS	5	Kostrewski et al.
526-73-8	§9.9 mg/m^3 (480 min)		5	(1997)
	25.0 mg/m^3 (480 min)		5	
	53.6 mg/m^3 (480 min)		5	
	94.8 mg/m^3 (480 min)		5	
Esters				
n-Butyl Acetate	70 mg/m^3 (20 min)	NS	24	Iregren et al. (1993)
123-86-4	350 mg/m^3 (20 min)		24	
	700 mg/m^3 (20 min)		24	
	1050 mg/m^3 (20 min)		24	
	1400 mg/m^3 (20 min)		24	
Ethylene glycol	14 mg/m^3 (240 min)	At rest	5	Groeseneken et al.
monomethyl ether	20 mg/m^3 (240 min)		5	(1987)
acetate 110-49-6	40 mg/m^3 (240 min)		5	
Alcohols				
Isopropanol 67-63-0	0 ppm (240 min)	NS	24	Van Thriel et al.
	34.9 ppm (240 min)		24	(2003)
	189.9 ppm (240 min)		24	
1-octanol 111-87-5	0 ppm (240 min)	NS	24	Van Thriel et al.
	6.4 ppm (240 min)		24	(2003)
	6.4 ppm (240 min)		24	
Ketones				
Methyl isobutyl	10 mg/m^3 (120 min)	50 W	11	Wigaues Hjelm et al.
ketone 108-10-1	100 mg/m^3 (120 min)		11	(1990)
	200 mg/m^3 (120 min)		11	
Ethers				
Methyl tertiary-	5 ppm (120 min)	50 W	2	Nihlén et al. (1998a)
Butyl Ether 1634-04-4	25 ppm (120 min)		2	
	50 ppm (120 min)		2	
	5 ppm (120 min)	50 W	2	Nihlén et al. (1998b)
	25 ppm (120 min)		2	
	50 ppm (120 min)		2	
	4.8 ppm (120 min)	50 W	2	Johanson et al.
	24 ppm (120 min)		2	(1995)
	49 ppm (120 min)		2	

Table 1 continued on next page

Human experimental exposure to solvents

TABLE 1: Continued.

Chemical class, Substance, CAS no.	Doses and exposure duration	Exercise	Number of subjects	References
Glycoethers				
Ethylene glycol monoethyl ether 110-80-5	10 ppm (240 min)	At rest	5	Groeseneken et al. (1986a)
	20 ppm (240 min)		5	
	40 ppm (240 min)		5	
	10 ppm (240 min)	At rest	5	Groeseneken et al. (1986b)
	20 ppm (240 min)		5	
	40 ppm (240 min)		5	
1-methoxy-2-propanol 107-98-2	15 ppm (300 min)	At rest	6	Devanthéry et al. (2002)
	50 ppm (300 min)		6	
	95 ppm (300 min)		6	
Halogenated hydrocarbons				
1,1,1-Trichloroethane 71-55-6	0 ppm (30 min)	NS	6	Gamberale et al. (1973)
	250 ppm (30 min)		6	
	350 ppm (30 min)		6	
	450 ppm (30 min)		6	
	550 ppm (30 min)		6	
1,1,1,2-tetrafluoro-ethane 811-97-2	1000 ppm (60 min)	NS	8	Emmen et al. (2000)
	2000 ppm (60 min)		8	
	4000 ppm (60 ppm)		8	
	8000 ppm (60 ppm)		8	
1,1,2-trichloro-1,2,2-trifluoroethane 76-13-1	1500 ppm (90 min)	NS	2	Stopps and McLaughlin (1966)
	2500 ppm (90 min)		2	
	3500 ppm (90 min)		2	
	4500 ppm (90 min)		2	
Tetrachloroethene 127-18-4	10 ppm (360 min)	NS	6	Völkel et al. (1998)
	20 ppm (360 min)		6	
	40 ppm (360 min)		6	
	100 ppm (240 min)	At rest	1	Fernandez et al. (1976)
	150 ppm (240 min)		1	
	200 ppm (240 min)		3	
	100 ppm (480 min)	At rest	5	Fernandez et al. (1976)
	150 ppm (480 min)		4	
	200 ppm (480 min)		3	
Trichlorethene 79-01-6	100 ppm (165 min)	NS	1	Stopps and McLaughlin (1966)
	200 ppm (165 min)		1	
	300 ppm (165 min)		1	
	500 ppm (165 min)		1	
	40 ppm (480 min)	NS	3	Bernauer et al. (1996)
	80 ppm (480 min)		3	
	160 ppm (480 min)		3	
	0 ppm (120 min)	NS	8	Vernon et al. (1969)
	100 ppm (120 min)		8	
	300 ppm (120 min)		8	
	1000 ppm (120 min)		8	
1,1,1,2,3,3,3-hepta-fluoropropane 431-89-0	1000 ppm (60 min)	NS	8	Emmen et al. (2000)
	2000 ppm (60 min)		8	
	4000 ppm (60 ppm)		8	
	8000 ppm (60 ppm)		8	

NS indicates not specified.

DISCUSSION

The bibliographical search initially enabled us to identify 206 selected papers, while the admission criterion led to the choice of 29 papers (Table 1). This figure represented 14.1 % of the total number.

Medline was effective in answering queries dating from the mid 1960s when the first research experiments were performed on human exposure by inhalation to volatile organic substances, most of them of industrial use.

Our survey excluded papers on epidemiological or field studies since it aimed at assessing exposures related to a single chemical substance. In fact the selected papers referred only to exposures to single substances in exposure chambers. This was deemed to be an essential prerequisite as it was the only experimental model capable of providing constant exposure levels, i.e. doses. All these articles have been published in journals on Occupational Medicine, Toxicology, Pharmacology, Environmental Health or Environmental Sciences.

Studies on exposure to *n*-hexane, xylene, cyclohexane, methyl formate, 2-butoxyethanol, cyclohexanol, acetone, methyl n-butyl ketone, methyl ethyl ketone, 1-metoxy-2-ethanol, 1-metoxy-2-propanol, trichlorofluoromethane, dichlorodifluoromethane, dichlorodifluoromethane, methylene chloride, methyl chloride and carbon sulphide were not included as they failed to meet the admission criterion.

The three-dose admission criterion was adopted since this is the most conservative of the possible way for identifying biphasic dose-response relationships. It should be noted that this criterion differs considerably from the one used by Calabrese and Baldwin (2001a) to build their database on hormesis. This diversity is due to the fact that these Authors did not include the human model in the construction of their data base, and also to the much more conservative admission criteria used as a result of the more numerous studies available. Our admission criterion is nonetheless adequate for our chosen aim which was to evaluate biphasic dose-response relationships, and not hormetic responses that must necessarily include the concept of No Observed Adverse Exposure Level (NOAEL).

All the studies reported in Table 1 refer to sessions in which a necessarily limited number of volunteers was exposed experimentally by inhalation to constant concentrations of chemicals. When investigated, the endpoints were represented by clinical or physiological parameters that were evaluated objectively or subjectively by the volunteers themselves. In this study blood, urinary concentrations, and expired air levels of unchanged substances or their metabolites were not considered as endpoints.

Hydrocarbons

Styrene

The aims of the study conducted by Stewart *et al.* (1968) were to evaluate the elimination of styrene in expired breath at different exposure

levels and to assess subjective and objective responses. In subjective responses such as irritation of the nose and throat, an increase in exposure resulted in a more severe response. Objective responses were evaluated through neurological examination, including the modified Romberg test. Abnormal responses were observed only at the highest dose. No evidence from the reported data indicated the presence of biphasic dose-response relationships.

Löf and Johanson (1993) studied the dose-dependent kinetics of inhaled styrene, by measuring the time course of styrene levels in capillary blood during and after exposure, and urinary mandelic acid after exposure. However, since the study did not include clinical and physiological endpoints, these Authors failed to observe biphasic dose-response relationships.

Toluene

Veulemans and Masscheelin (1978a, 1978b e 1979) studied exposure to toluene, but none of their studies evaluated a physiological or clinical endpoint. In fact the Authors reported only findings relating to individual respiratory uptake and elimination (1978a), to toluene blood levels during and after exposure (1978b) and to the relationship between individual toluene uptake and urinary hippuric acid excretion (1979).

In their study on the human response to toluene exposure, Andersen et al. (1983) studied twenty parameters concerning visual perception, vigilance, psychomotor functions, and higher cortical functions but failed to establish any type of biphasic dose-response correlation.

Kostrzewski and Piotrowski (1991) successfully investigated the possibility of using toluene concentrations in capillary blood as an exposure index for this solvent. This study provided toxicokinetic data such as the absorbed dose, retention in the respiratory tract and the kinetics of elimination of toluene in venous and capillary blood but not physiological or clinical endpoints.

We were not able to verify dose-response relationships in any of the admitted papers due to the lack of clinical or physiological endpoints.

Terpenes

Research performed on separate exposures to three natural terpenes, d-limonene (Falk *et al.* 1993), 3-carene (Falk *et al.* 1991) or the two enantiomers of α-pinene (Falk *et al.* 1990), report extensive data on the toxicokinetics, such as the concentration of each compound in capillary blood during and after exposure, urinary elimination of the unchanged terpene and health effects. Volunteers rated before, during, and after d-limonene exposure (Falk *et al.* 1993), the intensity of symptoms in eyes, nose, throat or airways (irritative symptoms), and headache, fatigue, quesiness, dizziness, a sense of intoxication, breathing difficulty and smell of solvent (CNS related symptoms). Neither irritative nor CNS-symptoms were reported by the volunteers for any of the exposure levels. The study

on exposure to 3-carene (Falk *et al.* 1991) was similar to that conducted on α-pinene with regard to exposure levels and the endpoints examined. In Figure 3 in the original study irritation ratings lead us to deduce a biphasic dose-response relationship, but a statistical evaluation cannot be made due to the absence of raw data. Experimental exposures to α-pinene (Falk *et al.* 1990) were performed in a similar way to that of *d*-limonene. However no dose-response relationships were revealed either for irritative or CNS-related symptoms.

Trimethylbenzenes

No physiological or clinical endpoint is reported in the study conducted by Kostrzewski and Wiaderna-Brycht (1995) on the toxicokinetics of 1,2,3-trimethylbenzene, one of the three isomers of trimethylbenzene. In fact the Authors report only findings on the absorption and elimination of the solvent from the blood and urinary excretion of its metabolite, 3,5-dimethylbenzoic acid. The same research team (Kostrzewski et al. 1997) subseqently extended this study to all three trimethylbenzene isomers, 1,2,3-trimethylbenzene, 1,3,5-trimetilbenzene and 1,2,4-trimethylbenezene, evaluating for each isomer the same parameters studied previously (Kostrzewski and Wiaderna-Brycht, 1995). Once again the lack of endpoints prevented dose-response relationships from being observed for these substances.

Halogenated hydrocarbons

Trichloroethene

Stopps and McLaughlin (1967) studied some psychophysiological functions following exposure to trichloroethene, administered first in increasing concentrations and subsequently in decreasing concentrations, manual dexterity. Necker tube test, card sorting, card sorting with an auxiliary test and dial display were used to evaluate the functions studied. Among these tests, the card sorting scores with auxiliary task and the dial display showed biphasic trend in relation to exposure. However it should be noted that the study was performed with a single subject and this made it impossible to carry out a statistical evaluation of the dose-response relationship.

Bernauer *et al.* (1996) evaluated the biotransformation of trichloroethene and the urinary excretion of its metabolites, trichloroacetic acid, trichloroethanol and mercapturic acids, but did not take physiological and clinical endpoints into consideration.

Exposures to four doses of trichlorothene (Vernon and Ferguson 1969) were designed in order to evaluate the visual-motor performance: Flicker Fusion, Howard-Dolman, Muller-Lyer, Steadiness and Purdue Pegboard tests. A biphasic trend was observed in the Flicker Fusion test,

but the statistical analysis provided by the Authors showed that the biphasic trend was not statistically significant.

Tetrachloroethene

The studies on tetrachloroethene carried out by Fernandez *et al.* (1976) report only data on pulmonary elimination of the solvent during and after exposure, while the work conducted by Völkel et al. (1998) focuses on the study of the biotransformation of tetrachloroethene and the urinary excretion of its metabolites such as trichloroacetic acid, dichloroacetic acid and *N*-acetyl-*S*-(trichlorovynil)-L-cysteine.

Freons

Emmem *et al.* (2000) reported on exposure to freon 1,1,1,2-tetrafluoroethane and 1,1,1,1,2,3,3,3-heptafluoroethane. Besides toxicokinetic data aimed at evaluating the elimination of these two substances from the blood, they also investigated endpoints in relation to cardiological (systolic and diastolic blood pressure, mean pulse rate) and respiratory effects (mean peak expiratory flow) measured during and after the exposure. The results showed neither alterations of any of the above-mentioned physiological parameters, nor biphasic dose-response relationship.

In their study on exposure to 1,1,2-trichloro-1,2,2-trifluoroethane, Stopps and McLaughlin (1967), using the same psychophysiological functions examined for trichloroethene (see above, Stopps and McLaughlin, 1967) in conjunction with the Short Employment Test-Clerical vigilance test, observed impaired psychomotory performance that followed a monotonic dose-response relationship.

Ethers

Methyl tertiary butyl ether

The studies of Johanson *et al.* (1995) on exposure to methyl tertiary butyl ether, besides evaluating this substance in exhaled air and in blood and its metabolite *tertiary* butyl alcohol in exhaled air during and after exposure, also assessed other effects such as: a) subjective ratings related to discomfort, irritative symptoms, CNS effects; b) eye measurements (redness, tear film break-up time, conjunctival damage, blinking frequency); c) nose measurements (peak respiratory flow, acoustic rhinometry and inflammatory markers in nasal lavage). No evidence was found to indicate biphasic response.

Nihlén *et al.* (1998a) measured toxicokinetic parameters such as the unmodified substance and its metabolite *tertiary* butyl alchool in exhaled air, blood and urine with the aim of providing a valid exposure index. Given the aims of this study, no clinical or biological endpoints were included. The same research team (Nihlén *et al.* 1998b) investigated the level of irritative symptoms, discomfort, and CNS effects by means of a

questionnaire. Ocular and nasal measurements, performed mostly at the highest exposure level, failed to produce any observable effect at the different exposure doses.

Ketones

Methyl isobutyl ketone

Data reported by Hjelm *et al.* (1990) on exposure to methyl isobutyl ketone referred not only to toxicokinetic parameters such as concentrations of the solvent in capillary blood, in urine before and after exposure, but also to subjective parameters such as irritative symptoms for eyes, nose and throat and CNS symptoms (headache, nausea and vertigo). All these symtoms were evaluated with a yes/no questionnaire, once before and five times during exposure. Other acute symptoms, such as local irritation or effects on the CNS – a total of 17 items – were assessed on a six point scale. This questionnaire was administered before exposure, five times during, and three times after exposure. The patterns of irritative symptoms and those affecting the CNS, assessed on a six point scale, were evaluated together and separately as a function of exposure. Moreover, a Mood scale was used and were also carried out two performance tests.

The yes/no questionnaire included the number of subjects that gave a positive answer as the dependent variable and this number increased monotonically with the exposure level. A biphasic pattern of CNS symptoms was observed using a six point scale on one single occasion after cessation of exposure, whereas, a biphasic trend was observed for irritative symptoms at two exposure concentrations during exposure. The aforementioned trends were deduced by observing Figures 5b and 5c in the original study. However, it should be noted that the failure to include a data table prevented us from making a statistical analysis of the findings. The Mood scale and the performance tests failed to reveal biphasic trends for the dose-response relationship.

Alcohols

Van Thriel *et al.* (2003) exposed chemically sensitive subjects to two concentrations of 1-octanol or isopropanol with a concurrent control; a considerable number of neurobehavioral tests were conducted separately for both the alcohols. “Well being” tests for tenseness, tiredness and annoyance were evaluated with a rating ranging from 1 to 7, while 29 acute symptoms were evaluated on a scale ranging from 0 to 5. Acute symptoms were grouped as follows: prenarcotic (4 symptoms), olfactory (4 symptoms), bad taste, (4 symptoms) respiratory (3 symptoms), nasal irritation (5 symptoms), eye irritation (7 symptoms) and other irritation (3 symptoms). Three neurophysiological tests included the Choice Reaction Time test, the Divided Attention task of the German attentional test battery and a vigilance test (a Mackworth clock test). Test timings

differed in accordance with typology. Interestingly, "well being" tests were evaluated at the pre-exposure time (1 measurement), during (9 measurements) and at the end of exposure (1 measurement). We studied the medians reported in Table 1 of the study by Van Thriel *et al.* (2003), extracting data relating to pre-exposure, at low and high levels. After exposure to 1-octanol, we observed a biphasic pattern for the dose-response relationship in the tiredness test. No further dose-response relationship with biphasic trend was observed for any of the other endpoints examined, either for 1-octanol or isopropanol.

Glycoethers

Ethylene glycol monoethyl ether

Groseneken *et al.* (1986a) studied toxicokinetic parameters such as the respiratory uptake and elimination of ethylene glycol monoethyl ether. They also investigated retention and atmospheric clearance, and some physiological parameters such as minute ventilation, respiratory frequency and oxygen consumption. However, none of the aforementioned parameters showed significant variations in relation to exposure levels. The same authors (1986b) carried out further studies on exposure to ethoxyacetic acid, the ethylene glycol monoethyl ether metabolite, during and after exposure. The authors focused their interest on the evaluation of some typical toxicokinetic parameters such as the half-life of the metabolite, its recovery after exposure as a function of the amount of ethylene glycol monoethyl ether inhaled, but failed to consider eventual biological endpoints.

1-methoxy-2-propanol

The study of Devanthery *et al.* (2002) on exposure of volunteers to 1-methoxy-2-propanol reports toxicokinetics data such as urinary excretion of free and total 1-methoxy-2-propanol during and after exposure, free 1-methoxy-2-propanol in blood during exposure and concentration of free 1-methoxy-2-propanol in expired air during and 30 min after exposure. No biological endpoints were studied, thus preventing observation of dose-response relationships.

Esters

n-butyl acetate

Iregren *et al.* (1993) studied the irritation and effects on CNS related to short exposures to *n*-butyl acetate. The irritant effects were studied for eyes, nose, throat, skin; breathing difficulty and sensation of bad smell were also studied. The CNS effects studied were headache, vertigo, nausea and tiredness. The subjects were asked to rate the intensity of these effects by a ten-point scale. For irritation to the throat, breathing difficulty and sensation of bad smell the ratings increased monotonically with

exposure. There were no significant effects on the CNS symptoms caused by exposure to this solvent.

Ethylene glycol monoethyl ether acetate

Groseneken *et al.* (1987) carried out studies on exposure to ethylene glycol monoethyl ether acetate, measuring numerous toxicokinetics parameters such as retention, atmospheric clearance, uptake, and respiratory elimination of ethylene glycol monoethyl ether, a metabolite of ethylene glycol monoethyl ether acetate, during and after exposure. Furthermore data concerning minute ventilation, oxygen consumption, respiratory frequency and heart rate were provided. None of the parameters studied revealed a biphasic dose-response relationship.

CONCLUSIONS

As far as we know, this study is the first attempt to investigate human experimental exposure by inhalation to volatile substances of industrial use with the specific aim of revealing biphasic dose-response relationships.

None of the studies shown in Table 1 were conducted to assess biphasic dose-response relationships. In fact, most of the studies were performed in the 1970s and '80s, when no one thought of testing the hypothesis of hormesis and the phenomenon was still practically unknown to most researchers in the toxicological field. Indeed, analysis of the publications reported in Table 1 show the following limitations:

- some studies do not include an endpoint
- statistical analysis is inadequate or totally lacking
- data tables that could be used to make an *a posteriori* analysis are lacking

We conclude that in the experimental exposure of humans to volatile substances, none of the studies reviewed demonstrates biphasic dose-response relationships.

However it should be pointed out that the lack of non-linear dose-response relationships in the studies reviewed could be due to the aforementioned limitations of the study designs. Naturally these limitations do not affect the intrinsic scientific value of either the selected or the admitted papers, conceived and performed mainly to evaluate the toxicokinetics of several industrial chemicals and their effects on humans.

From a statistical point of view, a study designed to test the hypothesis of a biphasic relationship should assess a linear and a polinomial function to explain dose-response observed data and to compare the goodness of fit indexes and residual distributions. At this regard we believe that a number of three doses selected in the range of levels lower than the occupational exposure standards would be consistent with the ethics of the experimentation.

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