Comments on Recommendation from Scientific Committee on Occupational Exposure Limits for 1,3-Butadiene

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CONTENTS

SUMMARY AND CONCLUSION

This document reviews the Recommendation on Occupational Exposure Limits for 1,3-butadiene (BD), developed by the Scientific Committee for Occupational Exposure Limits to Chemical Agents (SCOEL), document SCOEL/SUM/75E, June 1999.

Since ECETOC submitted its original criteria document on BD to SCOEL, significant new information has become available. This includes an update of the most relevant epidemiology study of styrene-butadiene rubber (SBR) workers, with improved cell type information and refined chemical exposure estimates including the addition of quantified exposure estimates for dimethyldithiocarbamate (DMDTC) which had been hypothesised as a potential cofounder.

The most critical finding was that BD exposures had been underestimated at least five-fold in the original report. Using the revised exposure estimates, even workers whose occasional high exposures were cumulatively equivalent to six months exposure at over 100 ppm showed little evidence of an increased risk of leukaemia and there was none in the absence of exposure to DMDTC.

New biomarker studies have demonstrated that BD is not genotoxic to workers at relevant occupational exposures.

The ECETOC Task Force believes that the new information is critical to the development of a sound assessment and should be taken into account when preparing a scientifically based Occupational Exposure Limit for BD. In the light of these new data, the risk estimates given in SCOEL/SUM/75E are considered to be unnecessarily conservative and should be recalculated to take account of the update of the SBR worker study.

1. INTRODUCTION

ECETOC welcomes the opportunity to comment upon the Recommendation on Occupational Exposure Limits for 1,3-butadiene (BD), developed by the Scientific Committee for Occupational Exposure Limits (SCOEL) to Chemical Agents, document SCOEL/SUM/75E, June 1999.

Since ECETOC submitted its original criteria document on BD to SCOEL (CEC, 1998), significant new information has become available which is critical to the evaluation of BD, including:

- An updated report on the University of Alabama in Birmingham (UAB) epidemiology study on BD. It includes improved cell type information and refined chemical exposure estimates for BD, styrene and dimethyldithiocarbamate (DMDTC).

- Biomarker studies in the Czech Republic and China which have examined both biomarkers of BD exposure and effects at relevant occupational exposures.

- Information on the potential role of DMDTC in leukaemogenesis within the styrene-butadiene rubber (SBR) industry.

These new data are provided and reviewed in this report.

2. SPECIFIC COMMENTS UPON THE TEXT OF THE RECOMMENDATION

In the following comments, reference is made to the text of the recommendation (SCOEL/SUM/75E, June 1999).

Page 3, Paragraphs 2-5

Data are cited on haemoglobin adducts in rats, mice and humans. Whilst such adducts can be detected, they are simply markers of exposure and do not demonstrate any significant biological effect of BD exposure. No changes in effect markers were found in two major new studies in which both biomarkers of exposure (metabolites in urine and haemoglobin adducts) and biomarkers of effects (chromosomal aberrations, mutations in T-cell lymphocytes) were examined. Details are given in Chapter 3.

Page 3, Paragraph 4

It is stated that Pérez *et al* (1997) reported that in two workers exposed to BD below 3 ppm (6.75 mg/m^3) the adduct level of the diepoxide was five times that of the control and about 70 times that of the monoepoxide adduct. If true, this would be a significant finding as the diepoxide, which is formed in mice at about 100 times the rate of rats and possibly accounts for the species differences in tumorigenic response between these rodents to BD, has not been detected in humans. However, the Pérez paper reports the finding of an epoxybutanediol-haemoglobin (not diepoxide) adduct in the two workers. Epoxybutanediol-haemoglobin adducts are not totally unexpected as epoxybutanediol is part of the main M1-metabolite excretory pathway in rats (and humans). As Pérez *et al* indicate, the epoxybutanediol can result from epoxybutene or from diepoxybutane. Our current understanding is that the former predominates in rats and humans.

Page 3, Paragraph 5

The differences in some of the early work on the potential genotoxicity of BD in man are highlighted. For example, new transitional epidemiological studies have shown no effect in a number of genetic markers in toxicology assays with workers exposed to 1 - 2 ppm BD. However, the study of Ward *et al* (1994) showed an association between exposure to sub-ppm levels of BD and *hprt* mutant frequencies. Overall, the weight of the evidence suggests that human exposure to BD at 1 - 2 ppm does not result in genotoxic effects. More information is given in Section 3.2.

Page 3, Last Paragraph, Continued on Page 4 (Epidemiology Review)

General Comments

The epidemiology review implies that findings are generally consistent across studies. On the contrary, while leukaemia deaths were elevated among SBR workers, 3 studies of BD monomer production workers have not found leukaemia mortality to be increased (Cowles *et al*, 1994; Divine and Hartman, 1996; Ward *et al*, 1996a). These studies are reviewed in Section 7.4.2 of CEC (1998). The SCOEL Recommendation fails to mention that leukaemia is not increased in monomer workers. Also, while the mortality of lymphosarcomas was elevated among BD production workers (Divine and Hartman,

1996; and Ward *et al*, 1996a) the larger, better characterised study of SBR workers by Delzell *et al* (1995, 1996, 2000), found no excess of non-Hodgkin's lymphoma (which includes lymphosarcoma) among SBR workers. Further, cumulative BD exposure was not associated with non-Hodgkin's lymphoma in monomer production workers (Divine and Hartman, 1996) or SBR workers (Delzell *et al*, 1995, 2000).

The lack of consistency in findings noted above was the basis for the IARC conclusion that the human evidence for carcinogenicity is limited (IARC, 1999). Additionally, the US-EPA Scientific Advisory Board (SAB) on BD concluded that it is improper to "lump" lymphomas and leukaemias together when evaluating consistency of findings across studies. Evaluating the cancers separately, the SAB concluded that the human data are inconsistent, and that the leukaemia excess among SBR workers could not be attributed solely to BD since these workers were exposed to several different chemicals (US-EPA, 1998).

Page 3, Last Paragraph, Line 10 "No Significant Increases …"

The description of results for the largest cohort of BD monomer workers (Divine and Hartman, 1996) focuses entirely on the Standard Mortality Ratio (SMR) findings rather than on the more powerful, informative internal regression analyses performed by the authors. The SMR does not measure the relationship between exposure and disease: it only assesses whether or not the frequency of deaths from a disease is increased compared to a reference population. In contrast, internal regression analyses specifically measure the relationship between exposure and disease and are thus critical to evaluating causality. The outcome of Divine and Hartman's internal regression analyses based on qualitative exposure measures showed no association between BD exposure and any lymphohaematopoietic cancer (LHC) type assessed, and this finding should be included in the SCOEL Recommendation.

Page 4, Line 2 from Top "whereas lymphosarcoma …"

The finding of Ward *et al* (1996a) that lymphosarcomas were increased among workers with > 2 years employment is cited, presumably to imply the existence of a dose-response trend. However, evaluating the data using < 2 years and ≥ 2 years categories does not constitute an adequate assessment of trend with duration of employment, especially given that three of the four cases worked for 39 months or less. Moreover, there is no mention of the fact that Ward *et al* (1996a) chose their duration categories solely on the basis of the distribution of the data. Finally, if two-year employment in BD monomer production were actually causally related to a 20-fold excess of lymphosarcoma, then such a finding should have been readily apparent among longer employed workers in the studies by Divine and Hartman (1996) and Delzell *et al* (1995, 1996, 2000).

Page 4, Line 12 from Top "An analysis of leukaemia mortality …"

The analysis of leukaemia mortality by cumulative exposure performed by Macaluso *et al* (1996) is said to be based on "a largely overlapping cohort of North American workers", i.e. overlapping with the cohort of Delzell *et al* (1995, 1996, 2000). However, the two cohorts are the same with the exception that Delzell *et al* excluded two plants with insufficient work histories for exposure estimation. Thus, use of the term "largely overlapping" is inappropriate and may give the reader a false impression as to the number of independent BD studies.

Page 4, Table "Cumulative Exposure … Relative Risk"

The ECETOC Task Force chose not to comment on the risk estimates table as the recent update of the UAB study provides a refined data set. The most critical finding was that BD exposures had been underestimated at least five-fold in the original report. Using the revised exposure estimates, even workers whose occasional high exposures were cumulatively equivalent to six months exposure at over 100 ppm showed little evidence of an increased risk of leukaemia and there was none in the absence of exposure to DMDTC. The reasons for the greater reliability of the new exposure estimate over the original are given in Section 3.1.

3. SIGNIFICANT NEW DATA

3.1 UAB Re-analysis - Exposure Estimates and Leukeamia Cell Type Information

In collaboration with an external industrial hygiene panel, Macaluso *et al* (2000) performed a detailed re-analysis of historical monomer exposures. A number of refinements were made, including verification of exposure model parameters with published literature (e.g. US Rubber Reserve Reports), further specification of exposure scenarios, performance of wind speed measurements (a critical model parameter), and obtaining further feedback from plant personnel on exposure scenarios and assumptions. The authors also further characterised peak exposures and revised the exposure estimation software to improve the examination of the effects of peaks, especially to calculate monomer ppm-year exposure estimates separately above and below peak levels. For BD the peak level was 100 ppm, and for styrene it was 50 ppm. This was not possible in the original study (Delzell *et al*, 1995,1996; Macaluso *et al*, 1996) and is a significant enhancement. Finally, Macaluso *et al* (2000) developed historical quantitative estimates of (dermal) DMDTC exposure in order to evaluate the hypothesis that increased leukaemia mortality among SBR workers may be due to confounding by DMDTC exposure (Irons and Pyatt, 1998). These new estimates are a significant improvement on those reported by Macaluso *et al* (1996) and Delzell *et al* (1995) for the following reasons:

- wind speed, a critical exposure model parameter, was directly measured rather than estimated as done previously;
- all the exposure scenarios were reviewed with industrial hygiene experts at all the plants in the study - not done for all scenarios previously;
- probabilistic exposure distributions were made using Monte Carlo simulations, not done previously to estimate exposure ranges;
- ppm-years above and below peak exposure threshold levels were separated, which was not previously possible;
- documentation of estimation methods was enhanced in terms of the assumptions, models, ranges and time periods for each job and task.

A comparison of estimates with measured data from one plant is also underway to verify the exposure estimation model. The same exposure estimating methodology used for BD and styrene (STY) was applied to develop DMDTC exposure estimates. Results from the exposure refinement (Macaluso *et al*, 2000) indicate that Delzell *et al* (1995) and Macaluso *et al* (1996) substantially underestimated both BD and STY exposures.

The re-analysis by Delzell *et al* (2000) of the relationship between leukaemia and BD exposure, based on the refined exposure estimates and additional information from medical/pathology reports on leukaemia cell types, indicates an association between cumulative BD exposure and leukaemia. This re-analysis has two important advantages over the original study published in 1996. These are:

- the quantification of (dermal) exposure to DMDTC, to assess its importance as a potential confounder, and
- the ability to assess the importance of high levels of exposure to BD.

For DMDTC, Poisson regression analyses adjusted for age, years of employment and STY and BD exposure indicated a higher magnitude of association compared with BD or STY, and all exposed categories showed either borderline or statistically significant increased risks (Table 1). However, the shape of the dose-response curve was irregular.

The relationship of leukaemia relative rates to both BD and DMDTC exposure is illustrated in Table 2. In this, workers were allocated to one of three exposure groups for both BD and DMDTC. In each case, the lowest exposure group comprised the unexposed and the lowest quintile of the exposed. The intermediate exposure group comprised the second and third quintiles of the exposed and the highest exposure group comprised the remaining fourth and fifth quintiles. This enabled the persons at risk and the leukaemia deaths to be allocated to one of the cells of a 3 x 3 matrix and the calculation of relative rates for each cell. The marginal rates, both adjusted and unadjusted, are also shown along with the p-values for the trends. Cross-classification of the data by BD and DMDTC exposure provided further information about risk associated with these two chemicals (Table 2).

Table 1: Relative Rate (RR) of Leukaemia Deaths, Adjusted for Age and Years since Hire, for Single and Multiple Exposure to BD, STY and DMDTC †

	L / PY[a]	Single exposure model	Multiple exposure models	
			BD + DMDTC[b]	BD + DMDTC + STY[c]
BD ppm-years[d]		**RR (95% CI)[e]**	**RR (95% CI)[h]**	**RR (95% CI)[i]**
0	7 / 48,139	1.0	1.0	1.0
> 0 - < 86.3	17 / 97,623	1.2 (0.5 - 3.0)	1.0 (0.4 - 2.4)	1.3 (0.4 - 4.3)
86.3 - < 362.2	18 / 60,114	2.0 (0.8 - 4.8)	1.2 (0.5 - 3.2)	1.3 (0.4 - 4.6)
≥ 362.2	17 / 28,540	3.8 (1.6 - 9.1)	2.2 (0.8 - 5.9)	2.3 (0.6 - 8.3)
STY ppm-years[d]		**RR (95% CI)[f]**	**RR (95% CI)[i]**	
0	5 / 34,749	1.0		1.0
> 0 - < 20.6	18 / 108,064	1.2 (0.5 - 3.3)		0.6 (0.1 - 3.7)
20.6 - < 60.4	18 / 55,103	2.3 (0.9 - 6.2)		0.8 (0.2 - 3.7)
≥ 60.4	18 / 36,499	3.2 (1.2 - 8.8)		0.8 (0.2 - 3.8)
DMDTC mg-years/cm[d, j]		**RR (95% CI)[g]**	**RR (95% CI)[h]**	**RR (95% CI)[i]**
0	13 / 98,781	1.0	1.0	1.0
> 0 - < 566.6	16 / 70,584	2.3 (1.1 - 4.8)	2.1 (1.0 - 4.6)	2.1 (0.8 - 5.4)
566.6 - < 1,395.1	15 / 25,544	4.9 (2.3 - 10.3)	3.9 (1.7 - 8.9)	4.1 (1.7 - 10.1)
≥ 1,395.1	15 / 39,507	2.9 (1.4 - 6.1)	2.0 (0.8 - 4.8)	2.2 (0.9 - 5.3)

† Adapted from Delzell et al, 2000

a Number of leukaemia cases / total number of person-years
b BD exposures corrected for DMDTC, DMDTC exposures corrected for BD
c All exposures corrected for the other two
d BD, STY and DMDTC models based on tertiles of leukaemia decedents' exposure distributions
e df = 50, χ^2 = 40, D = 46 (df, degrees of freedom; χ^2, Pearson chi-square; D, model deviance)
f df = 50, χ^2 = 38, D = 39
g df = 49, χ^2 = 34, D = 35
h df = 214, χ^2 = 136, D = 114
i df = 686, χ^2 = 383, D = 182
j The dermal DMDTC exposure estimation procedure yielded: (i) an estimate of the concentration of DMDTC in solution wetting the skin of the exposed worker (in mg/cm^3); (ii) an estimate of the skin surface exposed (in cm^2), and (iii) an estimate of the frequency and duration of exposure. Thus, the exposure intensity was estimated in (mg/cm^3) x (cm^2) = mg/cm.

Table 2: Relative Rate (RR) of Leukaemia Deaths, Adjusted for Age and Years since Hire, for Cross-classified BD and DMDTC Exposures[a] †

DMDTC mg-years/cm[b]		**BD ppm-years** 0 - < 38.7	38.7 - < 287.3	≥ 287.3	**Marginal DMDTC exposure[c] unadjusted for BD**	**Marginal DMDTC exposure[c] adjusted for BD**
0 - < 342.4	L / PY[e]	15 / 100,963	5 / 43,315	2 / 10,596	22 / 154,875	
	RR	1.0[f]	0.7	1.2	1.0[f]	1.0[f]
	95% CI	-	0.3 - 2.1	0.3 - 5.2	-	-
342.4 - <1222.6	L / PY[e]	2 / 8,237	11 / 20,727	5 / 7,691	18 / 36,655	
	RR	1.6	3.7	4.5	3.6	3.2
	95% CI	0.4 - 7.1	1.7 - 8.0	1.6 - 12.4	1.9 - 6.7	1.6 - 6.3
≥ 1,222.6	L / PY[e]	0[g] / 1,656	5 / 22,869	14 / 18,361	19 / 42,885	
	RR	-	1.4	4.4	2.9	2.1
	95% CI	-	0.5 - 3.9	2.1 - 9.1	1.5 - 5.3	1.0 - 4.4
Marginal BD exposure[d], unadjusted for DMDTC					p = 0.0002[h]	p = 0.047[h]
	L / PY[e]	17 / 11,0856	21 / 86,912	21 / 36,648		
	RR	1.0[f]	1.5	3.4		
	95% CI	-	0.8 - 2.9	1.8 - 6.4	p = 0.0003[h]	
Marginal BD exposure[d], adjusted for DMDTC						
	RR	1.0[f]	1.0	2.0		
	95% CI	-	0.5 - 2.1	0.9 - 4.3	p = 0.047[h]	

† Adapted from Delzell et al, 2000

a Three categories of each exposure variable specified as (i) no exposure plus the first quintile, (ii) the second and third quintiles and (iii) fourth and fifth quintiles

b The dermal DMDTC exposure estimation procedure yielded: (i) an estimate of the concentration of DMDTC in solution wetting the skin of the exposed worker (in mg/cm^3); (ii) an estimate of the skin surface exposed (in cm^2), and (iii) an estimate of the frequency and duration of exposure. Thus, the exposure intensity was estimated in $(mg/cm^3) \times (cm^2) = mg/cm$.

c Marginal DMDTC exposure refers to risk associated with DMDTC exposure, with data summed across all BD exposure levels

d Marginal BD exposure refers to risk associated with BD exposure, with data summed across all DMDTC exposure levels

e Number of leukaemia cases / total number of person-years

f Referent category

g Person-years excluded from analysis of cross-classified exposures

h p-value for trend

Table 2 shows that there was no evidence of a relationship between BD exposure and leukaemia risk in workers with low exposures to DMDTC (< 342.4 mg-years/cm) and this suggests that leukaemia risk is not due to BD alone. In workers with BD exposure > 38.7 ppm-years and exposure to DTDMC > 342.4 mg-years/cm, mortality from leukaemia was almost 4 times higher than that seen in workers with the same level of BD exposure but with the lowest cumulative exposure to DMDTC. Mortality from leukaemia in workers in the latter category was similar to that in the referent category and there were sufficient expected deaths from leukaemia to provide 95% power to detect an increase in leukaemia mortality of 2.6 fold (i.e. a much lower increase than seen in workers with BD exposure > 38.7 ppm-years and exposure to DTDMC > 342.4 mg-years/cm). Nevertheless, due to the lack of sufficient numbers of workers exposed to only BD or DMDTC, the data are insufficient to determine if the risk is due to BD alone, DMDTC alone, or only the combination of exposures.

The association between cumulative BD exposure and leukaemia was stronger for BD ppm-years due to exposure intensities ≥ 100 ppm than for ppm-years due to exposures at all concentrations (Table 3). Table 4 shows the leukaemia relative rate cross-classified for BD exposure intensities ≥ 100 ppm and exposure intensities below 100 ppm. There was no statistically significant trend between leukaemia mortality and BD ppm-years due to exposure intensities < 100 ppm, after adjustment for BD ppm-years due to exposure intensities ≥ 100 ppm ($p = 0.45$). However, there was a significant trend for increasing RR with increasing ppm-years due to exposure intensities ≥ 100 ppm when adjusted for exposure intensities < 100 ppm ($p = 0.01$). There were few person years in the BD exposed subgroup having no exposure to BD ≥ 100 ppm although there were no cases of leukaemia. However, over half the person years of observation of exposed workers were in the exposure category of < 46.5 ppm-years at exposure intensities of ≥ 100 ppm (i.e. up to 5.6 months of exposure), and there was only a slight increase in relative risk in this sub-group. The crude relative risk (unadjusted for age and years since hire) for workers in the subgroup was 1.16. The majority of these workers were exposed to greater than 1,000 ppm at some stage in their working life. If the analysis is further restricted to this subgroup then the crude relative risk only increases slightly to 1.4, and is still non-significant. Further information about the relative importance of exposure above 100 ppm is also provided by Macaluso (2000) who reported that the ratio of cumulative BD exposure to intensities ≥ 100 ppm to cumulative exposure to intensities < 100 ppm was considerably higher for leukaemia decedents (157:63 ppm-years) than for all decedents (56:30 ppm-years).

No associations between cumulative BD exposure and any specific leukaemia cell type or with non-Hodgkin's lymphoma were observed. Styrene showed no relationship with leukaemia or any other type of LHC when adjustment was made for other exposures.

Table 3: Relative Rate (RR) of Leukaemia Deaths, Adjusted for Age and Years Since Hire, for ppm-years Due to Overall BD Exposures and to Exposures ≥ 100 ppm and <100 ppm †

Exposure	L / PY[a]	BD only RR (95% CI)	BD + DMDTC RR (95% CI)
BD ppm-years due to all exposure intensities			
0	7 / 48,139	1.0	1.0
> 0 - < 86.3	17 / 97,623	1.2 (0.5 - 3.0)	1.0 (0.4 - 2.4)
86.3 - < 362.2	18 / 60,114	2.0 (0.8 - 4.8)	1.2 (0.5 - 3.2)
≥ 362.2	17 / 28,540	3.8 (1.6 - 9.1)	2.2 (0.8 - 5.9)
		df = 50, $\chi 2$ = 40, D = 46[b]	df = 214, $\chi 2$ = 136, D = 114
BD ppm-years due to exposure intensities ≥100 ppm			
0	7 / 65,596	1.0	1.0
> 0 - < 46.5	17 / 83,243	2.1 (0.9 - 5.1)	1.6 (0.6 - 4.0)
46.5 - < 234.3	17 / 57,586	2.8 (1.2 - 6.7)	1.7 (0.6 - 4.6)
≥ 234.3	18 / 27,990	5.8 (2.4 - 13.8)	3.6 (1.3 - 9.8)
		df = 50, $\chi 2$ = 38, D = 43	df = 213, $\chi 2$ = 141, D = 117
BD ppm-years due to exposure intensities < 100 ppm			
0	7 / 48,139	1.0	1.0
> 0 - < 37.8	17 / 10,8134	1.1 (0.5 - 2.7)	0.8 (0.3 - 2.0)
37.8 - < 96.3	17 / 39,886	2.8 (1.2 - 6.8)	1.8 (0.7 - 4.7)
≥ 96.3	18 / 38,256	3.0 (1.2 - 7.1)	1.9 (0.7 - 5.1)
		df = 49, $\chi 2$ = 45, D = 49	df = 213, $\chi 2$ = 146, D = 115

† Adapted from Delzell et al, 2000

[a] Number of leukaemia cases / total number of person-years

[b] df, degrees of freedom; $\chi 2$, Pearson chi-square; D, model deviance

Table 4: Leukaemia Relative Rate (RR), Adjusted for Age and Years since Hire, for BD ppm-years due to Exposure Intensities ≥ 100 ppm and for BD ppm-years due to Exposure Intensities < 100 ppm †

BD ppm-years due to exposure intensities < 100 ppm		**BD ppm-years due to exposure intensities ≥ 100 ppm** 0	>0 - < 46.5	46.5 - < 234.3	≥ 234.3	**Marginal BD ppm-years due to exposure intensities < 100 ppm**
0	L / PY[a]	7 / 48,139	- - - - - - - - No observations - - - - - - - -			7 / 48,139
	RR[b]	1.0				1.0
	95% CI					
> 0 - < 37.8	L / PY[a]	0 / 14,489	12 / 64,224	5 / 26,487	0 / 2,934	17 / 108,134
	RR	-[c]	1.4	1.3	-	1.1
	95% CI		0.5 - 3.5	0.4 - 4.1		0.5 - 2.7
> 37.8 - < 96.3	L / PY[a]	0 / 2,485	3 / 11,564	7 / 18,255	7 / 7,582	17 / 39,886
	RR	-	1.9	2.5	5.7	2.8
	95% CI		0.5 - 7.2	0.9 - 7.2	2.0 - 16.3	1.2 - 6.8
≥ 96.3	L / PY[a]	0 / 483	2 / 7,455	5 / 12,844	11 / 1,7474	18 / 38256
	RR	-	1.7	2.5	4.0	3.0
	95% CI	0.4 - 8.4	0.8 - 7.8	1.5 - 10.3	1.2 - 7.1	p = 0.45[d]
Marginal BD ppm-years[f] due to exposure intensities ≥ 100 ppm						
	L / PY[a]	7 / 65,596	17 / 83,243	17 / 57,586	18 / 27,990	
	RR	1.0	2.1	2.8	5.8	
	95% CI		0.9 - 5.1	1.2 - 6.7	2.4 - 13.8	p = 0.01[e]

† Adapted from Delzell et al, 2000

a Number of leukaemia cases / total number of person-years

b All RRs are adjusted for age and years since hire, only

c Dash indicates category not included in Poisson regression model

d p-value for trend in BD ppm-years due to exposure < 100 ppm, adjusting for BD ppm-years due to exposure ≥100 ppm, age and years since hire

e p-value for trend in BD ppm-years due to exposure ≥100 ppm, adjusting for BD ppm-years due to exposure < 100 ppm, age and years since hire

f Marginal BD exposure refers to risk associated with BD exposure, with data summed across all DMDTC exposure levels

3.2 Biomaker (of Exposure and Effects) Studies of SBR and BD Monomer Workers

Albertini (1999) conducted a transitional epidemiology study of biomarker responses in BD-exposed workers at two sites within an industrial complex near Prague in the Czech Republic. The cohort comprised 24 monomer production workers, 34 polymerisation (rubber) workers and 25 non-exposed controls (administrative workers). Ten separate full shift (8-hour) personal measurements were used to assess exposure; these were supplemented by 15-minute samples. The respective mean and maximum levels were reported as 0.29 ppm and 9.05 ppm in monomer workers, and 0.80 ppm and 17.74 ppm in SBR workers. In comparison, the mean and maximum levels in controls were 0.012 ppm and 0.057 ppm, respectively. The group differences were significant, although there were large inter- and intra-individual variations. Of the possible concomitant exposures to STY, benzene and toluene, only STY exposures (mean concentration of 2.4 ppm) were markedly higher in the polymerisation workers.

The biomarkers included by Albertini were those of exposure and dose (i.e. BD metabolites in urine, BD-haemoglobin adducts in blood, BD-DNA adducts in urine), of effect (sister chromatid exchanges, chromosomal aberrations, *hprt* mutations in T-cell lymphocytes by audioradiographic and clonal methodologies) and of susceptibility (glutathione S-transferase M1 and T1 genotypes). All analyses were conducted blind as to the sample origin and as to other measurements on that sample. There was a significant correlation of concentrations of the exposure biomarkers (metabolites of mercapturic acid butanediol [M1] and mercapturic acid butenol [M2], and haemoglobin adducts of monoepoxide and N-(2,3,4-trihydroxybutyl)valine [THBVal]) with measured airborne BD concentrations. For the effect biomarkers (somatic gene mutations, chromosomal changes or immunologic phenotype distributions) no significant relationships were observed. The susceptibility marker (GSH genotypes) showed no correlation with any biomarker response. The exposure assessment indicated good general control of exposures. As expected, exposures were higher in workers of the polymerisation plant than in workers of the monomer plant, which in turn were higher than controls.

Hayes *et al* (2000) evaluated genotoxic markers among 41 Chinese BD polymer production workers and 38 non-exposed controls matched for age and sex. All subjects completed a questionnaire regarding their work history, selected medical conditions, and tobacco use. Personal samplers were used to collect air in the breathing zone of the workers during their 6-hour work shift. Numerous grab samples at the breathing zone were also taken, along with canister (area) samples. The genotoxic endpoints assessed included glycophorin A (GPA), *hprt* mutant frequencies as determined by the T-cell cloning assay, and karyotype analysis by 6-banding to identify chromosomal abnormalities. Additionally, the role of genotoxic polymorphisms in GSTT1 and GSTM1 were evaluated in relation to genotoxicity among workers. Finally, M1 and M2 metabolites and THBVal haemoglobin adducts were assessed as measures of BD exposure.

The median exposure level among BD-exposed workers was 2 ppm (6-hour time weighted average), due largely to intermittent peak exposures. Compared with controls, BD-exposed workers had higher levels of THBVal adducts ($p < 0.0001$), and the adduct

levels correlated with air measurements (p = 0.03). Comparison of BD-exposed workers with controls did not indicate any differences in the frequency of un-induced or diepoxybutane-induced sister chromatid exchanges, aneuploidy as measured by fluorescence in situ hybridisation of chromosomes 1, 7, 8 and 12, GPA, or *hprt* mutant frequencies. Additionally, the higher THBVal level in exposed workers was not associated with increases in un-induced sister chromatid exchanges, aneuploidy, GPA, or *hprt* mutations. Among BD-exposed workers, the GSTM1 and GSTT1 genotype did not predict M1 formation, THBVal adducts, un-induced sister chromatid exchanges, aneuploidy, or mutations in GPA or *hprt* genes. There was a modestly higher lymphocyte (p = 0.002) and platelet count (p = 0.07) in BD-exposed workers compared with controls, and lymphocytes as a percentage of white blood cells were moderately correlated with greater THBVal levels (r = 0.32, p = 0.07) in BD-exposed workers. However, the authors indicate that the mechanism and significance of this modest increase are unknown and earlier studies showed no haematological effects (Checkoway and Williams, 1982; Cowles *et al*, 1994).

The authors concluded that, overall, the study demonstrated exposure to BD in these workers, by a variety of short-term and long-term measures, but did not show specific genotoxic effects, at the chromosomal or gene levels, related to the exposure.

3.2.1 Evaluation

The absence of genotoxic effects seen in Czech workers by Albertini (1999) is consistent with results from the smaller, independent study of BD-exposed workers in China conducted by Hayes *et al* (2000). These findings appear to be inconsistent with earlier studies by Legator *et al* (1993), Ward *et al* (1994, 1996b), Au *et al* (1995) and Šrám *et al* (1998). The more recent studies observed no increase in the frequency of chromosomal aberrations or *hprt* mutations, even though Albertini used two different methodologies, i.e. audioradiographic and clonal assays. These different methodologies had been suggested as a possible explanation for the lack of genotoxic response reported by Tates *et al* (1996) using the cloning assay and the elevation in *hprt* seen by Ward *et al* (1996b) using the audioradiographic assay. Another possible explanation was that the inconsistency in study findings was due to genotype variation. However, this does not appear to hold true since new findings of Albertini (1999) and Hayes *et al* (2000) showed no evidence of GSH genotype correlation with *hprt* mutation frequency. Although the mutant frequency was higher than expected in the administrative workers in the Czech cohort and similar to those in exposed workers, the biomarkers confirmed the absence of BD-exposure in the controls. The reason for the elevated background in this study remains unclear. Other studies by Sorsa *et al* (1996) and Tates *et al* (1996) also showed no evidence of genotoxicity.

3.3 Co-Exposure to DMDTC and Possible Confounding Effects

In the text of the SCOEL Recommendation no mention is made of co-exposure of SBR workers to DMDTC and possible confounding effects associated with such exposure, despite the presence of biological evidence to support this. Unfortunately, as noted above (Section 3.1.1), there were insufficient numbers of workers exposed only to DMDTC

in the SBR worker study (Delzell *et al* 1995, 1996, 2000) to test adequately the co-exposure or confounding hypothesis. However, there was an association, similar or greater than that with BD, of leukaemia with DMDTC exposure (Table 1). The lack of a relationship between BD exposure and leukaemia risk in workers with low exposures to DMDTC (< 342.4 mg-years/cm) suggest that leukaemia risk is not due to BD alone. As noted earlier, the risk of leukaemia in workers in the two highest BD cumulative exposure categories and with the lowest exposures to DMDTC, was less than 1/3 that seen in workers with the same exposure to BD but co-exposed to higher levels of DMDTC. Thus, the hypothesis raised by Irons and Pyatt (1998) of confounding exposure in the SBR worker study (Delzell *et al* 1995, 1996, 2000) cannot be dismissed.

Subsequent studies (Appendix A) have shown that sodium DMDTC is readily absorbed through the skin of mice and can alter the metabolism of BD monoepoxide. Administration of BD monoepoxide alone did not significantly affect the protein carbonyl content of liver microsomes of mice, but following co-administration of DMDTC to mice, the microsomal protein carbonyl content was increased to 70% above control. DMDTC exposure alone resulted in a 40% increase (Witz, 1998; Bird *et al*, 1999). This carbonyl accumulation is suggestive evidence for inhibition of the aldehyde pathway by DMDTC resulting in accumulation of a metabolite containing an aldehyde group. Further, Elliott and Ashby (1980) showed that DMDTC increased the level of glutathione-S transferase. Since 3-butene-1,2-diol is a major metabolite in man and rat (when compared to the mouse) due to the higher epoxide hydrolase in these species, it is possible that an adduct containing a free aldehyde group and thiophene moiety is formed through the interaction of glutathione with the 1-hydroxy-3-butene-2-one, the oxidation product of 3-butene-1,2-diol, potentiated by increased GST activity. Inhibition of aldehyde dehydrogenase by DMDTC would result in the accumulation of this adduct and/or transport of this adduct into the bone marrow stem cell through inactivation of surface aldehyde dehydrogenase. Thus, the above information provides a supportive hypothesis for the biological plausibility of DMDTC as a potential confounding or effect modification factor in the SBR studies.

Further details on the biological activity of DMDTC are given in Appendix A.

4. CONCLUSION

The UAB research team has newly refined the historical exposure estimates for BD and STY in its epidemiological study of SBR workers (Delzell *et al*, 2000; Macaluso *et al*, 2000). These refined exposure data show that BD exposures were underestimated at least 5-fold in the original report (Delzell *et al*, 1995,1996; Macaluso *et al*, 1996). A re-analysis of the SBR data using the refined exposure estimates and additional information on leukaemia cell types showed an association between estimated cumulative BD exposure and leukaemia. However, even workers whose occasional high exposures were cumulatively equivalent to six months exposure at over 100 ppm showed little evidence of an increased risk of leukaemia and there was none in the absence of exposure to DMDTC.

It is widely accepted that it is inappropriate to combine lymphomas and leukaemias when evaluating consistency of findings across studies (US-EPA, 1998; IARC 1999). Consequently, only one study has provided internally consistent evidence of a relationship between BD exposures and leukaemia in SBR workers. This finding has yet to be confirmed in other study populations i.e. monomer workers. This lack of confirmation together with the information on the biological activity of the process confounder DMDTC, and the lack of genotoxic effects in comprehensive studies of BD exposed workers, calls into question whether BD alone is the responsible aetiologic agent.

The ECETOC Task Force believes that the new information is critical to the development of a sound assessment and should be taken into account when preparing a scientifically based Occupational Exposure Limit for BD. In the light of these new data the risk estimates given in SCOEL/SUM/75E are considered to be unnecessarily conservative and should be recalculated to take account of the update of the SBR worker study.

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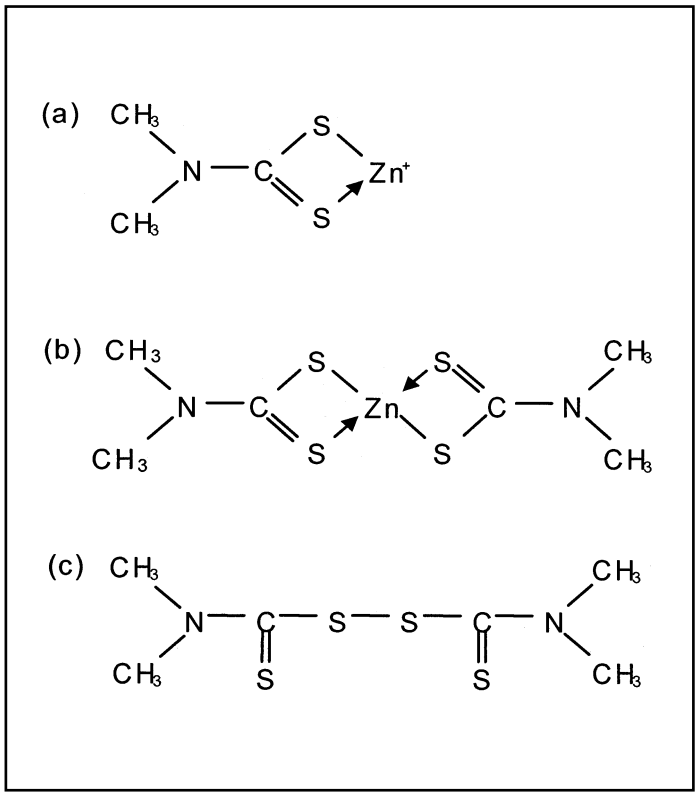
APPENDIX A. BIOLOGICAL ACTIVITY OF DMDTC - A POTENTIAL CONFOUNDER IN THE INTERPRETATION OF LEUKAEMIA MORTALITY IN THE SBR INDUSTRY

The dithiocarbamates (DTCs) represent a class of thiono-sulphur compounds that are chemically related to the thiuram disulphides, the oxidised form of the DTCs. The structures of dimethyldithiocarbamate (DMDTC) and its disulphide (tetramethylthiuram disulphide, TMTD) are shown in Figure A.1. The disulphides may be reduced to the DTCs *in vivo* by, for example, glutathione (Rannung and Rannung, 1984).

DTCs and thiurams are used in a number of applications, including reaction modifiers in rubber production, fungicides, and therapeutic control of chronic alcohol abuse. These compounds have complex biological properties, including inhibition of several enzyme systems, toxicity to the haematopoietic and immune systems, and mutagenicity.

DMDTC, in common with other members of this class, forms complexes with metal ions through chelation of the two sulphur atoms. The order of chelation is dependent upon the relative abundance of DMDTC and the metal ions, with DMDTC forming a 1:1 complex at low concentrations and a 2:1 complex at higher concentrations (Figure A.1).

Figure A.1: Structures of Zinc DMDTC, as the (a) 1:1 and (b) 2:1 Complexes, and (c) DMDTC Disulphide (TMTD)



The order of chelation affects the bioavailability and activity of DMDTC, and is postulated to account for non-standard dose-responses in toxicity evaluations (Rannung and Rannung, 1984; Hemavathy and Krishnamurthy, 1988).

Studies have been conducted with a range of DMDTC-metal complexes, as well as salts and the disulphide. Most have used either zinc DMDTC (ziram) or TMTD (thiram), and this discussion will be limited to these two forms except where necessary to add other important information.

A.1 Biological Activity of DMDTC

A.1.1 Interaction of DTCs with Metabolic Pathways

DTCs and thiurams are potent inhibitors of cytochrome $P_{450}2E_1$ and aldehyde dehydrogenase. The therapeutic basis for the use of tetraethylthiuram disulphide ("Antabuse"), the dimer of diethyldithiocarbamate, in the behavioural treatment of alcoholism is through its inhibition of aldehyde metabolism and clearance.

Interference in the Metabolism of BD

DMDTC may interact with the metabolism of BD in a number of ways, and the most obvious possibility is for it to inhibit the oxidation of BD to the toxic mono- and diepoxides (Irons and Pyatt, 1998). However, 3-butenal and crotonaldehyde have been identified as minor metabolites of BD by mouse liver microsomes and human myeloperoxidase (Elfarra *et al*, 1991; Duescher and Elfarra, 1992, 1993; Sharer *et al*, 1992). Therefore, an alternative possibility is that DMDTC may inhibit the further metabolism of these intermediates. DMDTC has recently been shown to increase the level of aldehyde substances in the livers of $B6C3F_1$ mice exposed to BD monoepoxide by 70%. The elevation of protein carbonyls did not occur with the epoxide alone and was increased by only 40% when DMDTC was administered alone. These data indicate that DMDTC modulates BD metabolism, and alters the balance in favour of aldehyde substance(s), possibly through inhibiting the further metabolism of BD-derived aldehydes (Witz, 1998; Bird *et al*, 1999).

A.1.2 Mutagenicity of DMDTC

In Vitro Studies

The mutagenicity of DMDTC has been assessed in both prokaryotic and eukaryotic cells in the presence and absence of metabolic activation. Both zinc DMDTC and TMTD increased revertants in *Salmonella* and *Escherichia* strains which are deficient in excision repair, but elicited only weak or equivocal responses in strains sensitive to frameshift mutations (Seiler, 1973; Shirasu *et al*, 1977; Hedenstedt *et al*, 1979; Moriya *et al*, 1983; Rannung and Rannung, 1984; Crebelli *et al*, 1985, 1992; Tinkler *et al*, 1988). Zinc DMDTC also induced chromosomal aberrations in human lymphocytes, CHO cells, and CHEL cells in the presence of a metabolic fraction, but gave only an equivocal response in the mouse lymphoma gene mutation assay (Tinkler *et al*, 1988; Mosesso *et al*, 1994). TMTD induced chromosomal aberrations in the Chinese hamster cells (Mosesso *et al*, 1994), unscheduled DNA synthesis and SCE in human lymphocytes in the presence of a metabolic fraction (Perocco *et al*, 1994) and mutations in the *hprt* locus of Chinese hamster V79 cells (Paschin and Bakhitova, 1985).

In Vivo Studies

Groups of five male Swiss albino mice were treated with zinc DMDTC (350, 700 or 1,050 mg/kgbw p.o.), administered as two doses 24 hours apart. Bone marrow samples were taken 6 hours after the final administration, and were assessed for micronuclei by counting 3,000 PCEs and a similar number of NCEs. Treatment resulted in an increase in the incidence of cells with micronuclei at all doses (Hemavathy and Krishnamurthy, 1988). In another study, groups of ten male and female $B6C3F_1$ mice were treated with zinc DMDTC by i.p. injection. Males were administered 2.5, 5 or 10 mg/kgbw, whilst females were given 5, 10 or 20 mg/kgbw (20 mg/kgbw caused 60% mortality in males). Bone marrow samples were taken 24 hours and 48 hours after treatment and 2,000 PCEs were scored for each animal to assess the incidence of micronucleated cells. DMDTC increased the incidence of cells with micronuclei only in males at 24 hours, and this was statistically significant only in the intermediate dose group (Crebelli *et al*, 1992).

A limited report indicates that zinc DMDTC (100 mg/kgbw) increased micronuclei in the bone marrow of mice by 110% or 580% when administered by gavage or i.p. injection respectively (Kurinny and Kondratenko, 1972). TMTD has consistently given positive results in several micronucleus assays (Kurinny and Kondratenko, 1972; Dulout *et al*, 1982; Paschin and Bakhitova; 1985; Crebelli *et al*, 1992).

Zinc DMDTC has also been reported to induce chromosomal aberrations in leukocytes of occupationally exposed workers (Pilinskaya, 1970), germ cells of male mice (Hemavathy and Krishnamurthy, 1988) and in *Drosophila* (Hemavathy and Krishnamurthy, 1989). TMTD induces chromosomal aberrations and sperm head abnormalities in mice (Zdzienicka *et al*, 1982; Prasad *et al*, 1987).

Zinc DMDTC (125, 250 or 500 mg/kgbw, higher doses were acutely toxic) was administered to rats and livers were removed either 2 hours or 16 hours later for assessment of unscheduled DNA synthesis. Isolated hepatocytes showed a dose-related increase in tritiated thymidine incorporation in both the nucleus and cytoplasm, although the net nuclear grain count was not increased by treatment (Tinkler *et al*, 1988).

DMDTC has shown mixed responses in *in vivo* studies. Although one reason for this may be the variability in purity of the samples used (Crebelli *et al*, 1992), protocol differences may also be a deciding factor. Studies use different strains of mouse, routes of exposure, and forms of DMDTC. Depending on any of these factors, the acute toxicity of DMDTC may limit the doses that can be administered. Thiram has given results that are more consistent across studies, presumably because the lower acute toxicity of this substance allows greater consistency in the administration of biologically active doses. However, it does appear that DMDTC is mutagenic in a number of test systems.

Interaction between DMDTC and BD

Further evidence of DMDTC activity in the bone marrow was reported in a study into the interaction of BD and DMDTC in male and female $B6C3F_1$ mice. DMDTC (sodium salt, 300 mg/kgbw, percutaneous), both alone and in conjunction with BD, increased the incidence of PCEs in the bone marrow and blood, whereas BD alone was without effect. BD increased the incidence of micronucleated PCEs in the bone marrow and

blood, whereas DMDTC alone was without effect. However, when DMDTC was administered prior to BD exposure, the incidence of micronuclei was lower than that induced by BD alone (Exxon Chemical Company, 1998). This limited study indicates that DMDTC is absorbed through the skin and affects the bone marrow, as well as interacting systemically with BD.

A.1.3 Carcinogenicity of DMDTC

A carcinogenicity bioassay conducted in mice for four DMDTC derivatives (zinc and iron complexes, disulphide and the dimethylamine salt) showed none of them to induce tumours over a period of 18 months (Innes *et al*, 1969). In contrast, zinc DMDTC induced tumours in 7/20 rats, compared to 1/46 controls over a period of 22 months (Andrianova and Alekseev, 1970). However, the reporting of these studies is limited, as were the exposure periods. It is unlikely that they could be taken as definitive demonstration of the carcinogenic potential of DMDTC. Indeed, IARC has considered the evidence to be equivocal (IARC, 1976).

A.1.4 Immunotoxicity

T-lymphocyte maturation, signalling and activation is mediated by nuclear factor kB (NF-κB), which is a member of the Rel family of transcription factors. Dissociation of NF-κB from its inhibitory protein and translocation to the cell nucleus activates a number of genes, including interleukin-2, involved in immune function and inflammation.

DMDTC disrupts intercellular signalling between primary human $CD4^+$ T-lymphocytes *in vitro*, as evidenced by inhibition of TNF-α-mediated NF-κB activation (Pyatt *et al*, 1998). Studies with BD mono- and diepoxides showed them not to inhibit T-lymphocyte activation (Irons and Pyatt, 1998).

A.2 Exposure to DMDTC in the SBR Industry

The retrospective study of workers in the SBR industry conducted by Delzell and co-workers (Delzell *et al*, 1995; Delzell *et al*, 1996) has been updated to provide detailed quantitative estimates of historical DMDTC exposure, which confirm the opportunity for exposure to DMDTC in the SBR industry. There is also anecdotal evidence for systemic exposure. Occupational exposure to DMDTC, which is a potent aldehyde dehydrogenase inhibitor, was associated with alcohol intolerance in SBR workers (Exxon Chemical Company, 1998; Irons and Pyatt, 1998).

In vivo experimental work conducted into the biological fate of DMDTC shows that DMDTC is systemically available following exposure via the skin (Exxon Chemical Company, 1998). As such, exposure may result from manual handling of DMDTC, or polymerisation products containing residual DMDTC, as well as by inhalation (Irons and Pyatt, 1998).

A.3 Summary

DMDTC has been shown to modify the metabolism of BD *in vivo*, changing the path or rate of metabolism from that which would be favoured in its absence. Furthermore, DMDTC (or structurally related compounds) has been shown to be active within the target tissues for suspected leukaemogenic effects. The most recent UAB study examined the relationship between leukaemia and quantitative estimates of exposure for DMDTC, BD, and styrene. The results indicate an approximately 5-fold statistically significant risk associated with exposure to DMDTC and BD together, as well as individual associations for these two chemicals.

Therefore, it is plausible that BD may not be the (sole) causative agent in the aetiology of leukaemia found in studies of SBR workers. The use of DMDTC coincided temporally with the induction of leukaemia in SBR workers, and this agent may modify the toxicokinetics or toxicodynamics of BD and its metabolites in the body.

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