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Adopted as the Journal of the Faculty of Occupational Medicine of the Royal College of Physicians of London

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ISSN 1351 0711

Published by BMJ Publishing Group, and printed in England on acid free paper by Thanet Press Limited, Margate, Kent

Analysis of a petrol plume over England: 18–19 January 1997

F Welch, V S G Murray, A G Robins, R G Derwent, D B Ryall, M L Williams, A J Elliott

Abstract

***Objectives*—About 7000 tonnes of unleaded petrol were discharged into the English Channel after a tanker collision off Ostend on Saturday 18 January 1997. The petrol evaporated and the vapour plume was carried across the central part of England to Wales, resulting in reports of unidentified odours, and irritation of the eyes, skin, and upper respiratory tract. This work uses this incident to show how marine and atmospheric dispersion modelling together with routine air quality monitoring can assist in identifying hazards to the population at risk from chemical incidents.**

***Methods*—Public health surveillance and results from environmental sampling were compared with the behaviour of the plume as predicted by computer modelling.**

***Results*—The predicted plume path and dispersion were shown to correlate well with the results from surveillance and environmental analysis.**

***Conclusions*—There is a need for public health professionals to interact with medical toxicologists, atmospheric and marine scientists and engineers, and other environmental experts in managing events of this nature.**

(*Occup Environ Med* 1999;56:649–656)

Keywords: petrol spill; environmental monitoring; dispersion modelling

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Accepted 10 June 1999

Incident summary

From 1232 hours on Sunday 19 January, the National Poisons Information Service (London) at the Medical Toxicology Unit (MTU), received 14 enquiries from across the country requesting information and advice relating to a strong petrol like smell. The enquiries came from accident and emergency departments, public health and environmental health offices, police, and the media. These enquiries concerned people experiencing irritation of the eyes, mucous membranes, and upper respiratory tract. The cause of these symptoms was not immediately clear. Some local sources suggested that local industry or aircraft jettisoning aviation fuel over the area could be responsible. However, the wide spread origin of the queries suggested another cause.

Discussions with accident and emergency departments, public health in Norfolk, Lincolnshire, Derbyshire, Nottinghamshire, Yorkshire, and Wirral, police stations in Nottinghamshire, Derbyshire, and Cheshire, coastguard operations room in Great Yarmouth and Dover, the Marine Pollution Control Unit, the Departments of Transport, Health and the Environment, and other National Poisons Information Services throughout the country led to the (as it then was) hypothesis that the cause was a collision in the English Channel on Saturday 18 January 1997. At 1500 hours on that day, the tanker, Bona Fulmar, collided in thick fog with the chemical carrying tanker, Teotal. The accident occurred 32 miles north west of Ostend in the French part of the Channel. Figure 1 shows the location of the incident.

It was reported that 7000 tonnes of unleaded petrol were spilt.[1] Crew from the Marine Pollution Control Unit flew over the area on the morning of Sunday 19 January and reported that they could not see a slick. At the request of the Chemical Incident Response Service (CIRS), a water sample was taken from the spill site on the Sunday at about 1500. This confirmed that unleaded petrol had been spilt.

With weather data for the period 18–19 January, the United Kingdom Meteorological Office used its long range pollution dispersion modelling system, NAME,[2] to plot the likely course of the petrol vapour plume (fig 2). This indicated that the plume was first carried up the North Sea before the change in wind direction which resulted in it subsequently being swept across the central part of England to Wales.

The aim of the work described in this paper was to illustrate how marine and atmospheric dispersion modelling together with routine air quality monitoring can assist in identifying hazards to the population at risk from chemical incidents.

Methods

SURVEILLANCE

In this context, surveillance describes the monitoring of the population for possible end effects. The NAME predictions showed that in travelling up the North Sea and then over the

Figure 1 Map showing the site of the spill.

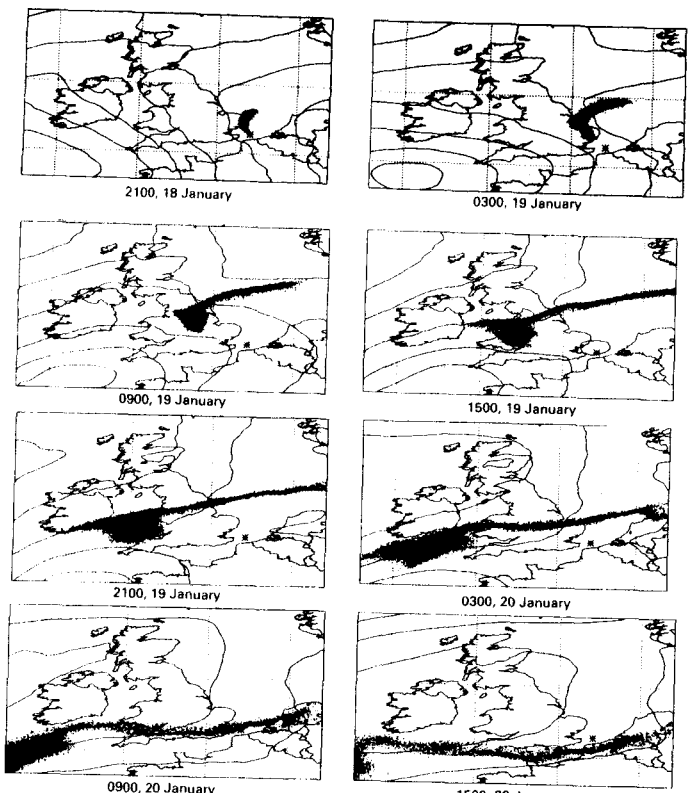

Figure 2 A sequence of NAME model output at 6 hour intervals for a steady release of 9 hours duration. Results from NAME output assuming a 9 hour release.

central part of England, the plume's first land fall was in Norfolk. Enquiries to the local consultant in communicable disease control subsequently established that no ill effects or odours had been reported there. Where available, chief emergency planning officer, police, and hospital admission logs were requested for review for areas possibly affected by the plume. Leeds, Liverpool, Birmingham, Cardiff, and Derbyshire were included as well as Norfolk. No accident and emergency departments reported cases to the National Poisons Information Service (London) or Chemical Incident Response Service over the seven day surveillance period, which lasted until Satruday 25 January.

ENVIRONMENTAL SAMPLING

Marine Pollution Control Unit

The Marine Pollution Control Unit (Maritime and Coastguard Agency) arranged for the collection of a sea water sample from the location of the spill on the Sunday afternoon at about 1500. The content of volatile hydrocarbons was determined by a "headspace analysis" of the vapour above the sample in a closed container. The analysis was carried out by the National Environmental Technology Centre at AEA Technology, Culham. Flame ionisation detector based gas chromatography (GC/FID) was used, this being the preferred method for analysis of such hydrocarbon mixtures. (Brian Jones, personal communication)

UK Hydrocarbon Monitoring Network

The UK Hydrocarbon Network (funded by the Department of Environment, Transport and the Regions) is a series of 13 continuous monitoring sites throughout the country where concentrations of hydrocarbons are measured. The network is run by the National Physical Laboratory. The analytical technique is again based on GC/FID methods, although the network procedures are automatic and operate on an hourly cycle. The concentrations of 26 hydrocarbons are measured in an air sample collected over a 35 minute period in each cycle. The data are transmitted automatically to a central unit from where they are relayed to public information services such as CEEFAX, Teletext, and the web site of the DETR. Data displayed on these media and on a free telephone line are updated every hour.

Considerable fluctuations in the concentration of hydrocarbons at a fixed site are not unusual. These could be due to a local source—such as a petrol station, a factory, or motor vehicle exhausts. Many hydrocarbons, those identified in the headspace analysis included, are present both in petrol and vehicle exhaust fumes, so an increase in concentration could simply imply an increase in traffic or that the wind has passed through a polluted urban area. Hydrocarbon concentrations due to emissions in motor vehicle exhausts need to be subtracted to identify contributions from other sources. An ideal tracer for traffic emissions is a hydrocarbon which is present in the exhaust but not in the fuel: 1,3-butadiene is just such a compound,[3] and changes in the concentrations of other hydrocarbons relative to 1,3-butadiene were used to identify contributions from sources other than traffic emissions.

Hydrocarbon concentrations from each site in question were taken from the United Kingdom air quality internet website.[4] The concentration of each chemical, as identified by the head space analysis, was plotted against time. In most urban areas across the United Kingdom, the predominant source of most volatile organic compounds is traffic, so the next step in the analysis was to remove this component. The hourly mean concentrations were first normalised by division by their monthly mean. The normalised results showed the enhancement over average concentrations due to meteorological variations and changes in emission patterns or source behaviour. The normalised 1,3-butadiene concentrations were then subtracted from the results for all other pollutants to remove the contribution due to traffic exhaust emissions. Any excursions from zero in the resultant plot were therefore taken to indicate contributions from sources other than traffic exhaust.

Non-traffic related concentrations of 1,3-butadiene may be due to several causes—such as local spills and industrial processes—which include the manufacture of synthetic rubber goods such as tyres. 1,3-Butadiene is formed during the combustion of petrol.[5]

Carbon monoxide has been used in the past as the indicator for traffic in analyses of this kind. This has some practical limitations in that most of the hydrocarbon monitoring sites are not at the same location as the associated carbon monoxide monitoring sites. The Birmingham East site however records carbon monoxide and hydrocarbons so in this case the monitoring results could be used to examine the relative performance of both means of identifying traffic emissions. An analysis based on carbon monoxide was found to yield almost identical results to the one based on 1,3-butadiene, lending confidence to the use of 1,3-butadiene.

DISPERSION MODELLING

Marine dispersion modelling

The period taken for the spill to evaporate was unknown and this introduced some uncertainty both in the prediction of its dispersion at sea and in the treatment of the resulting vapour plume in the atmosphere. In temperate conditions, components with a boiling point <200°C, such as petrol, tend to evaporate fully within the first day, with a half life (the time taken for 50% of the oil to disappear from the sea surface) of a few hours.[6] No slick was visible when the Marine Pollution Control Unit flew over the location of the accident on the morning of 19 January, about 18 hours after the event.

The oil and chemical spill model, EUROSPILL,[7] was used to simulate the motion of the oil slick on the sea surface. The spill simulations used surface winds provided by the Meteorological Office's operational numerical weather prediction models.

Atmospheric dispersion modelling

The main atmospheric dispersion modelling effort was undertaken with the Meteorological Office's NAME model.[7] Like EUROSPILL, this too is a Lagrangian dispersion model driven by output from the Office's weather prediction models. Loss of plume material due to wet and dry deposition is included in the NAME model but was ignored in the present work.

The use of the three dimensional wind fields ensures that all relevant dispersion phenomena are included in the NAME calculations. Plume dispersion over short periods, typically an hour or so, can normally be approximated by spread and dilution about a straight trajectory. However, over longer periods the developing wind field generally causes the plume to deviate from its initial direction of travel and complex plume trajectories can result. Changes in wind direction with height cause plume elements at different altitudes to follow different trajectories and the resulting lateral spread can be more significant than the mixing due to turbulence. Vertical mixing then transfers the enhanced lateral spread to all levels.

Calculations were undertaken for different assumed evaporation periods from 3 to 24 hours, with a constant emission rate over the evaporation period. This treatment of the spill as a constant source strength for a fixed duration was clearly a great simplification. Comparisons of resulting predictions of concentrations with results of environmental monitoring were then used to estimate the most likely evaporation period.

Some simple dispersion calculations were also carried out with one of the long range transport models included in the R91 range of models.[8] The likely ranges of hourly averaged concentrations at 500 km from the source were calculated for different spill evaporation times.

Results of the headspace analysis

Hydrocarbon	*Concentration (ppb)*
Benzene	4.5
Toluene	2.0
Ethylbenzene	0.80
(m+p)-Xylene	0.88
o-Xylene	2.6
Unidentified	>1500

Data supplied by Brian Jones, personal communication.

Results

SURVEILLANCE

A review of the chief emergency planning officer and police logs from the Derbyshire area (Mary Newlands, personal communication) showed that reports of odours were logged from 0714 for over 2 hours on Sunday 19 January 1997. The reports ranged in description, with the odour source being described as chemicals, aviation fuel, glue, cellulose, or simply a funny sweet smell. At this stage there was no mention of the tanker accident on the previous day. The Chief Emergency Planning Officer contacted by telephone possible local sources such as chemical plants which were all able to confirm they were not the source. Review of the log from the Derby Royal Infirmary showed that there was no obvious increase in attendances for people with symptoms likely to be due to exposure to the passing plume. Some police who were working during the incident reported sore throats, and streaming eyes and noses (Mary Newlands, personal communication).

Contact with other consultants in communicable disease control and poisons units in other areas showed that strange odours and symptoms were reported from Leeds, Chester, Liverpool, Birmingham, and Cardiff. Many of the reports came from Environmental Health Officers investigating the incident. There were no reports of odours or symptoms from Norfolk although the plume probably passed over this area between about 2100 GMT on 18 January and 0300 GMT on 19 January when it was still relatively concentrated.

HEAD SPACE ANALYSIS

Results of the head space analysis are summarised in the table. The FID output indicated

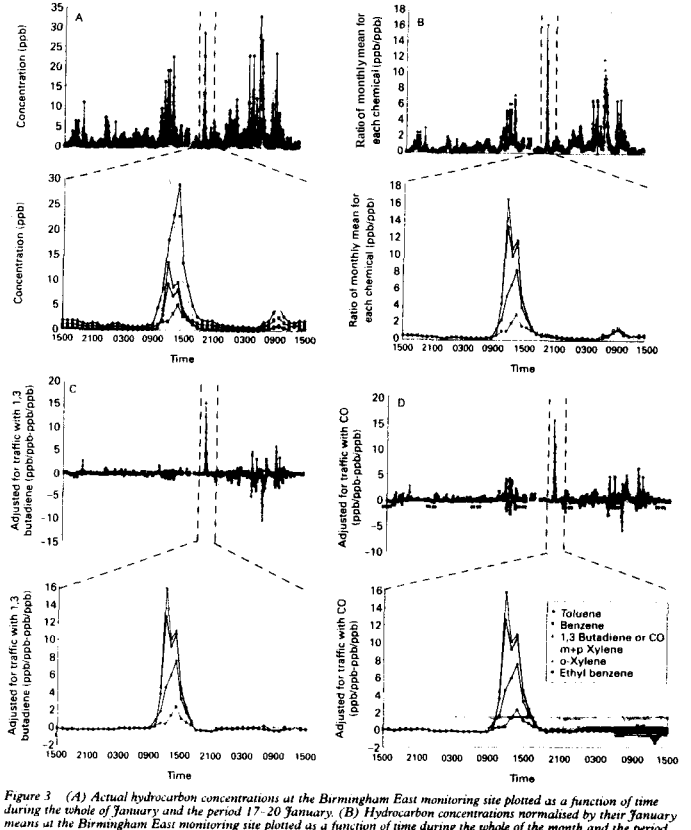

Figure 3 (A) Actual hydrocarbon concentrations at the Birmingham East monitoring site plotted as a function of time during the whole of January and the period 17–20 January. (B) Hydrocarbon concentrations normalised by their January means at the Birmingham East monitoring site plotted as a function of time during the whole of the month and the period 17–20 January. (C) Normalised hydrocarbon concentrations with traffic components removed at the Birmingham East monitoring site plotted as a function of time during the whole of the month and the period 17–20 January. (D) As (C) with carbon monoxide rather than 1,3 butadiene as the indicator.

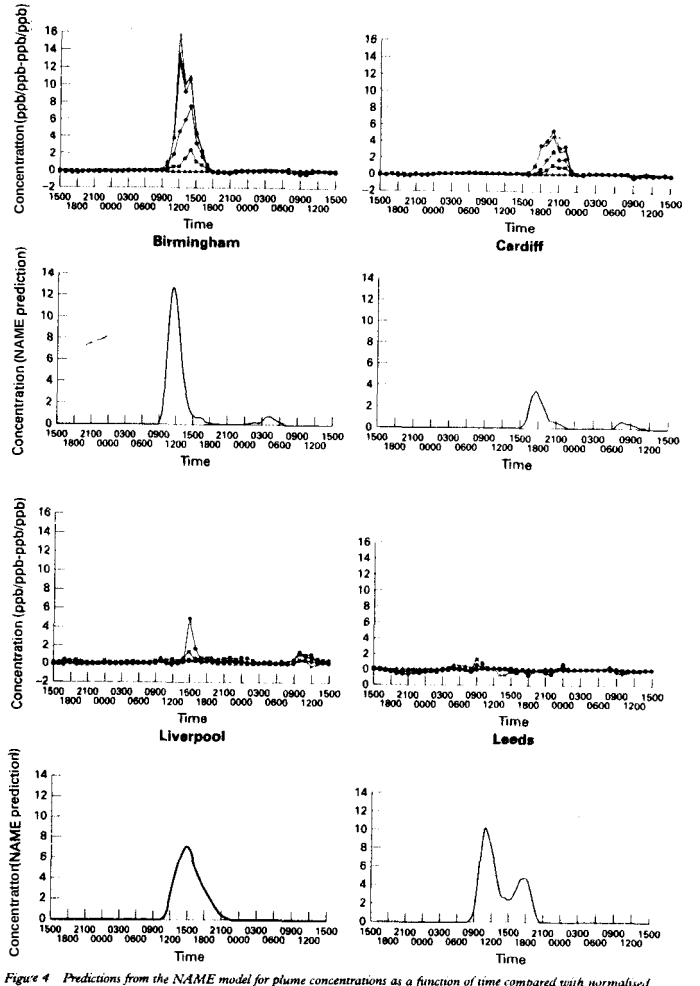

Figure 4 Predictions from the NAME model for plume concentrations as a function of time compared with normalised hydrocarbon concentrations with traffic components removed at the selected monitoring site plotted as a function of time during the period 17–20 January.

that the unidentified compounds were probably C5 and C6 hydrocarbons. Separation and identification of these compounds was impossible as several unidentified peaks coeluted or partially coeluted in the part of the chromatogram where aliphatic C5 and C6 hydrocarbons usually elute. Some of the unidentified peak shapes suggested that they may be polar compounds, possibly oxygentated hydrocarbons. A ketone smell did seem to dominate the aroma of the sample although there may possibly have been esters present (Brian Jones, personal communication). Ketones and esters are both oxygenated hydrocarbons.

HYDROCARBON NETWORK RESULTS

The output from most network sites showed some form of activity at the time of interest. However, a clearer picture emerged once the traffic generated component was removed by use of the techniques already described. Figure 3 illustrates the process for output from the Birmingham monitoring site. The actual hydrocarbon concentrations are plotted as a

function of time during the whole of January and the period of interest in figure 3 A. Numerous peaks are apparent, including one of about 8 hours duration on the 19 January. The same results are also shown normalised by the appropriate monthly average. This shows that the peak concentrations of ethylbenzene, *m+p* xylene and *o*-xylene on 19 January were about 15 times greater than their monthly averages (fig 3 B). The traffic component was then removed by subtraction of the normalised 1,3 butadiene concentrations, leaving the results shown in figure 3 C. This reduced the baseline to zero throughout most of the month, with a clear peak in all the other hydrocarbons plotted. Similar results were obtained when carbon monoxide was used as the traffic indicator, althought the baseline becomes noisier (fig 3 D). Finally, analysis of the monitoring site output over a 12 month period subsequently confirmed that the peak concentrations found on 19 January were indeed exceptional (figs not included).

Figure 4 shows the normalised hydrocarbon levels with traffic components removed at the selected monitoring site plotted as a function of time 17–20 January at Birmingham East, Cardiff, Liverpool, and Leeds.

DISPERSION MODELLING

Marine dispersion

The displacement of the oil slick by wind and tide during the first day after the spill was calculated by the EUROSPILL model to be no more than a few km.[9] On the scale of the area affected by the gas cloud this was negligible.

Atmospheric dispersion

The output from the NAME model was a mapping of fluid particle number densities as a function of time after the start of the release. A sequence of results at 12 hour intervals is shown in figure 2 for a steady release of 9 hours duration. The predictions show that the plume first travelled in a northerly direction, making first landfall over Norfolk at around 2100 GMT on 18 January. The cloud of polluted air then became stretched out in a roughly east-west direction, although its centre drifted slowly to the north west. Material from the early part of the release remained over the North Sea but from about midnight polluted air from the North Sea part of the release began to cross central England, reaching Wales by about 1200 on 19 January. On the next day the whole plume, which by this time was very dilute, drifted slowly to the south.

An estimate of the likely duration of release was determined by comparing predicted concentrations for 3, 6, 9, 12, and 24 hour duration releases with observations at Birmingham, London, Cardiff, Bristol, and Liverpool.

A peak was predicted at Liverpool for all releases, implying that the material detected here was released soon after the accident (within 3 hours). Peaks at Birmingham and Cardiff were only predicted for releases of ≥9 hours, while peaks at Bristol and London were predicted for releases of 12 and 24 hours respectively. As peaks were identified at Liverpool, Birmingham, and Cardiff, but not at London or Bristol, this suggests a release time of about 9 hours.

Assuming 140 tonnes of benzene was released over a 9 hour period, the model predicts maximum concentrations in the range 1–10 μg/m³ over the north of England, between 18 and 24 hours after the start of the release. This is consistent with concentrations of benzene found.

The results of simple dispersion modelling with the long range transport section of the R91 group of models[8] give benzene concentrations in the range of 3–7.5 μg/m³ at a distance of 500 km.

Discussion

SURVEILLANCE

Odours and associated effects were widely reported in the Midlands, although not in other regions where they were anticipated, such as Norfolk. Nevertheless, the geographical spread of reports suggests that a local source was not likely to be responsible. Several questions remain unanswered and are discussed later.

ENVIRONMENTAL SAMPLING

The headspace analysis showed that the sample consisted mainly of C5 and C6 hydrocarbons plus toluene, which is a C7 hydrocarbon, xylenes and ethylbenzene, both C8s. The headspace sample itself was not taken until Sunday 19 January, some time after the initial spill, and so the results cannot be considered as representative of the actual emissions at the time of the spill. Although the more volatile components had probably evaporated by this time, the results do confirm that the tanker contained a light petroleum fraction such as gasoline.

The odour which alerted most people was described as a sweet, solvent type smell and was probably due to oxygenated hydrocarbons. Ketones are sweet smelling and commonly used as solvents for lacquers, paint, and nail varnish removers.[10 11] Esters too have a strong sweet odour, and are present in natural flavours and used in artificial flavourings.[11]

Figure 3 shows that the excess hydrocarbon concentrations in Birmingham persisted for about 8 hours and that during this period concentrations varied in a relatively smooth manner. This suggests a wide plume from a distant source for some duration. Signals from local spills or emissions would most likely be characterised by a short duration and highly fluctuating concentrations.

Birmingham has been used to illustrate the analysis procedure because this location gave the clearest signal of all the sites examined. The analysis clearly showed the hydrocarbon peak at the Cardiff monitoring site, and possibly also at Liverpool (fig 4) although at lower concentrations than at Birmingham. During the period of interest no *o*-xylene results were available at Cardiff, although the concentrations of the remaining compounds were in the same relative proportions as at Birmingham.

Other sources of hydrocarbons, including 1,3 butadiene, near the Liverpool site (as well as traffic) might have affected both the monthly average and the concentrations found on 19 January, weakening any conclusions which might be drawn.

Analysis of the data from Belfast, Bristol, Edinburgh, London Eltham, UC London, Harwell, Leeds, Middlesborough, and Southampton failed to find unambiguous signs of the gas cloud.

The concentrations of other hydrocarbons monitored by the Network were examined for the Birmingham site. This showed the following hydrocarbons, all components of petrol, to be evident in the plume: *i*-butane, *n*-butane, trans but-2-ene, but-1-ene, cis but-2-ene, *i*-pentane, *n*-hexane, toluene, ethylbenzene, *m+p*-xylene and *o*-xylene.

The following were not detected: ethane, ethylene, propylene, propane, acetylene, *n*-pentane, 1,3-butadiene, trans pent-2-ene, cis pent-2-ene, 2-methylpentane, and *n*-heptane.

This confirmed that the increase in hydrocarbons was not traffic related as ethylene, propylene, acetylene, and 1,3-butadiene have all been identified as components of motor vehicle exhaust but not petrol. Toluene, ethylbenzene, *o*-xylene, *m+p*-xylene, *n*-butane, and *i*-butane have been clearly identified as present in both motor vehicle exhaust and petrol. However, their concentrations relative to benzene were considerably different from the values normally associated with urban air pollution, which again confirmed that the plume was not traffic related.

There is no obvious component of petrol that could have created a distinct odour at the levels of dilution indicated by dispersion modelling or implied by the measurements. Strictly speaking we cannot confirm that the odours were a result of the petrol spill. Although the possibility of chemical changes within the plume cannot be ruled out, we note that the accident occurred in winter and photochemical oxidation even over the long travel times involved is unlikely to have been efficient. Furthermore, summertime photochemical oxidation is not usually considered as a source of odorous organic compounds from evaporated petrol.

The hydrocarbon monitoring sites do not measure concentrations of oxygenated compounds. The analytical method, VOCAIR, requires the air to be dried by the use of a plastic membrane before analysis. This means that polar compounds (which include ketones, esters, and other odorous substances) are removed.

DISPERSION MODELLING

Concentrations of benzene predicted by the NAME and R91 models were of similar magnitude to those detected in the plume by the monitoring network. The odour threshold for benzene is 15 mg/m³,[12] typically a factor of 10^3 larger than the predicted concentrations. Although individual people are able to detect odours well below this threshold,[12] these results suggest that the plume was too dilute for benzene odours to be detectable. This conclusion probably applies to all primary emissions from the spill. The odour thresholds of some oxygenated compounds are much lower but their production in sufficient quantities would need to be explained, as does the cause of the other symptoms reported.

The output from the NAME model for a 9 hour emission, located plume material in space and time which matched the anecdotal and surveillance evidence. Moreover, the results showed that material from the later part of such an emission was carried across the midlands, something which did not arise with an emission of shorter duration. Figure 4 compares predictions for a 9 hour emission with monitoring site data. This shows close agreement on plume arrival and persistence times, and the variation of concentration with time, at the two sites where unambiguous plume signals were detected, Birmingham and Cardiff. This is also true of Liverpool, but not Bristol, despite its geographical proximity to Cardiff.

The NAME model suggests that Leeds was affected, but the monitoring suggests that this was not the case. Leeds was at the northern limit of the simulated plume's reach.

The deduction of an emission duration of about 9 hours is perhaps surprising in view of the volatility of petrol, but it provides a match between the predicted behaviour of the plume and the monitoring observations.

Conclusions

The reports and anecdotal evidence, together with the atmospheric modelling and measurement evidence suggests that the odours and reported symptoms experienced across Trent, Merseyside, the midlands, and south Wales were likely to have been due to the spill of petrol from the *Bona Fulmar*. This means that significant effects were detected at distances of the order of ≥500 km from the scene of the accident. In this case there were no serious adverse health effects, but had the meteorology been slightly different, taking the plume directly across south east England, or had the tanker contained chemicals which had similar physical properties, but substantially more serious toxicological effects, then the consequences could have been quite different. Although this was an unusual incident, it is not unique and the possibility of similar events cannot be discounted. The International Tanker Owners' Pollution Federation web site[9] lists further details on oil spills.

At the time, no regard was given to those who may have been affected at large distances from the spill. Marine and meteorological modelling, together with environmental surveillance and monitoring, have been shown to be powerful tools for analysis of the event. These are clearly also suitable management tools, both in terms of short and long range impacts. Collaboration between the professions and organisations involved should be encouraged to provide an efficient overall approach.

Several aspects of the incident have yet to be explained. The substances actually responsible for the odours and other symptoms have not been established. The plume dilution was too great for primary emissions to have been responsible.

It was found that 1,3-butadiene is an effective tracer for traffic pollution when used in conjunction with the technique described.

Many have contributed to the unravelling of this incident. In particular we would like to thank Brian Jones, AEA Technology; Peter Woods, National Physical Laboratory; Mary Newlands, consultant in communicable disease control, South Derbyshire Health Authority; and Paula MacDonald, consultant in communicable disease control, Communicable Disease Unit, Public Health Laboratories, Chester. The views expressed by MJW are his own and do not represent those of the Department of the Environment, Transport, and the Regions. RGD and DR acknowledge the Public Meteorological Service research and development programme of the Meteorological Office for their funding and support. Financial support to FW from the Engineering Physical Science Research Council as part of the EngD in Environmental Technology at the University of Surrey is gratefully acknowledged.

1 Anon. Weather key factor in North Sea collision and gasoline spill. *Oil Spill Intelligence Report* 23 January 1997;2.
2 Maryon RH. Modelling the long range transport of radionuclides following a nuclear accident. *Nucl Energy* 1994;33: 119–28.
3 Ye Y, Galbally IE, Weeks IA, *et al*. Evaporative emissions of 1,3-butadiene from petrol-fuelled motor vehicles. *Atmospheric Environment* 1998;32:2685–92.
4 Department of the Environment, Transport, and the Regions. *Air quality information for the UK, 1998*. http://www.aeat.co.uk/netcen/aqarchive/auto.html
5 Department of the Environment, Scottish Office, Welsh Office. *The United Kingdom national air quality strategy, March 1997*. London: The Stationery Office, 1997. (CM 3587.)
6 International Tanker Owners Pollution Federation. *Oil spills from tankers*. http://www.itopf.com/
7 Elliott AJ. EUROSPILL - oceanographic processes and NW European shelf databases. *Marine Pollution Bulletins* 1991; 11:548–53.
8 Jones JA. *A model for long range atmospheric dispersion of radionuclides released over a short period*. Harwell: National Radiological Protection Board, 1981. (Report No R124.)
9 Elliott AJ. Simulations of atmospheric dispersion following a spillage of petroluem at sea. *Spill Science and Technology Bulletin* 1999;5:39–50.
10 Fessenden RJ, Fessenden JS, eds. *Organic chemistry, 3rd ed*. New York: Brooks/Cole, 1986.
11 Ebbing DD, ed. *General Chemistry*. Boston: Houghton Mifflin, 1990.
12 Valentin FHH, North AA, eds. *Odour control: a concise guide*. Stevenage, Herts: Department of Industry. Warren Spring Laboratory Report, 1980.

Reproductive endocrine effects of acute exposure to toluene in men and women

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Accepted 15 June 1999

Abstract

***Objectives*—Despite observation of adverse reproductive effects of toluene, including alterations of serum gonadotropins (luteinising hormone (LH) and follicle stimulating hormone (FSH)) in humans, little is known of the mechanism of toxicity. The hypothesis was tested that toluene acutely suppresses pulsatile gonadotropin secretion by measuring LH and FSH at frequent intervals during controlled exposure to toluene.**

***Methods*—Women in the follicular and luteal phases of the menstrual cycle and men were randomised to inhale filtered air with or without 50 ppm toluene through a mouthpiece for 3 hours (19% of the OSHA permissable exposure limit). Blood was sampled by intravenous catheter at 20 minute intervals for 3 hours before, 3 hours during, and 3 hours after exposure. Plasma LH, FSH, and testosterone were measured. Pulse amplitude, pulse frequency, and mean concentrations of LH and FSH for each of the 3 hour periods before, during and after exposure to toluene versus sham exposure were calculated with the ULTRA pulse detection program and compared by analysis of variance (ANOVA) with repeated measures.**

***Results*—In men mean concentrations of LH showed a significant interaction ($p<0.05$) between exposure and sampling period, with a greater LH decline during exposure to toluene than sham exposure. However, there was no concomitant effect on testosterone concentrations. The LH pulse frequency of women in the luteal phase showed a trend towards a significant interaction between exposure and sampling period ($p=0.06$), with a greater decline in pulse frequency during exposure to toluene than sham exposure. There were no other significant effects of exposure to toluene.**

***Conclusions*—Three hour exposure to 50 ppm toluene did not result in abnormal episodic LH or FSH secretion profiles, however, subtle effects on LH secretion in men and women in the luteal phase were found. The clinical relevance of these effects is unclear, indicating the need for further study of reproductive function in exposed workers.**

(*Occup Environ Med* 1999;56:657–666)

Keywords: solvents; luteinising hormone; follicle stimulating hormone

Recommended human occupational and environmental exposure limits to solvent chemicals have been based primarily on studies designed to detect global effects such as encephalopathy. Most of these studies were not designed to detect subtle alterations in hypothalamic-pituitary-gonadal function that might adversely affect the reproductive health of millions of exposed workers. The aromatic solvent toluene, which is widely used in such diverse manufacturing industries as shoes, textiles, electronic components, and plastics exemplifies this dilemma. Despite several studies suggesting adverse reproductive effects of exposures to mixed solvents[1–5] and a few studies implicating toluene specifically,[6–8] very little is known about the mechanisms by which toluene might disrupt reproductive function.

Although relatively little research has been undertaken to examine the human reproductive effects of solvent compounds, occupational exposure to organic solvents has been reported to be associated with decreased fecundability,[1 4] infertility,[5] dysmenorrhoea, and irregular menstrual cycles.[1 2 6] Similarly, studies of reproductive endocrine function in male rotogravure workers exposed exclusively to toluene reported lower luteinising hormone (LH), follicle stimulating hormone (FSH), and testosterone concentrations.[7 8] The findings of diminished LH, FSH, and testosterone in these studies suggest a primary suppressive effect of toluene on LH and FSH secretion at the pituitary level or suppression of hypothalamic gonadotropic releasing hormone (GnRH) secretion or both. A central effect of toluene on reproductive function is further supported by neuroanatomical studies in both humans and animals showing that toluene preferentially distributes to the brainstem, including the hypothalamus.[9] Moreover, in female rats exposure to toluene reduces GnRH content in the preoptic area of the hypothalamus,[10] and in male rats it alters hypothalamic noradrenaline (norepinephrine),[11 12] the primary neurotransmitter involved in regulation of GnRH secretion.[13]

Previous investigations of the effects of toluene on gonadotropin secretion have been limited by the use of single measurements of gonadotropin concentrations after exposure. As these hormones are known to be secreted episodically, with significant minute to minute fluctuations in concentration particularly for LH, only large differences in hormone concentrations are likely to be detected with single samples.[14–18] The ability to detect differences in gonadotropin concentrations among groups can be greatly enhanced by multiple

sampling,[14] which has the additional advantage of enabling the detection of LH pulse frequency, which directly reflects GnRH pulse frequency.[19]

The purpose of this study, therefore, was to use the technique of repeated blood sampling to test the hypotheses that (*a*) acute exposure to toluene suppresses episodic gonadotropin secretion in men and women, (*b*) that this effect is mediated through a central hypothalamic effect suppressing GnRH pulse frequency, and (*c*) to determine whether such effects on the hypothalamic-pituitary axis occur at concentrations within permissible standards.

Methods

STUDY DESIGN

The study used a controlled, blinded, interventional experimental design, with reproductive hormone concentrations (LH, FSH) determined before, during, and after exposure to inhaled toluene. The research protocols were approved by the University of Washington Human Subjects Review Committee. All subjects gave written, informed consent. Blood sampling was conducted under the supervision of the Clinical Research Center of the University of Washington Medical Center.

RECRUITMENT OF SUBJECTS

Men and women aged 19–45 were recruited from the University and surrounding community through posters and advertisements in the printed media. Eligibility criteria included (*a*) absence of clinically important chronic disease, (*b*) no active exposure to solvents, (*c*) no cigarette smoking or other substance misuse, (*d*) absence of menstrual or fertility disorders or history of adverse pregnancy outcomes, (*e*) no current pregnancy, confirmed by measurement of serum human chorionic gonadotropin (HCG) 1 day before the study day, (*f*) no hormonal contraceptive use, (*g*) use of reliable non-hormonal contraceptive method.

DETERMINATION OF MENSTRUAL CYCLE IN WOMEN

A menstrual history was elicited to establish the regularity and timing of menstrual cycles. Subjects had to have regular menstrual cycles during the 6 months preceding enrolment to the study. Mid-follicular phase was defined as 5–9 days after the onset of menses for a 28 day cycle.[20] Mid-luteal phase was defined as 5–9 days before the expected first day of the next cycle for a 28 day cycle.[20] For cycles not 28 days in duration, the luteal phase was assumed to be 14 days in duration, with the appropriate adjustments made for the follicular phase.[21]

EXPERIMENTAL PROTOCOLS TO ASSESS EFFECTS OF EXPOSURE TO TOLUENE ON PULSATILE GONADOTROPIN SECRETION

Men or regularly cycling women aged 19–45 were randomised for exposure to a mixture of toluene and filtered air (n=6 mid-luteal phase, n=5 mid-follicular phase, n=5 men) or to filtered air alone (n=5 mid-luteal, n=5 mid-follicular, n=5 men) for 3 hours as described below. The stages of the menstrual cycle were chosen because there is no diurnal variation in LH or FSH during these stages and no mid-cycle preovulatory gonadotropin surges.[16 18] Although there is no diurnal variation in FSH secretion in men, about 10% of men have increased LH pulse frequency during sleep.[17] Therefore, samples were taken from awake subjects during normal waking hours.

Venous blood samples were collected from a forearm catheter into heparinised Vacutainer tubes (Becton-Dickinson, Franklin Lakes, NJ, USA) after clearing the line of saline. Samples were collected at 20 minute intervals for 9 hours (3 hours before onset of exposure, 3 hours during exposure, and 3 hours after the end of exposure). Sampling periods of 3 hours were judged to be sufficient to determine hormonal pulse frequency. A time interval of 20 minutes between samples has been shown to permit detection of gonadotropin pulses in humans.[20] Subjects were given breakfast and lunch and were free to move about between blood sampling, except during the exposure period when they remained seated in a chair.

Samples were refrigerated for up to 9 hours at 4°C, then were centrifuged, and plasma was collected and stored at -80°C until subsequent LH and FSH fluoroimmunoassay. Toluene concentration in whole blood drawn at the end of the exposure period was measured on the day of the exposure for subjects from both groups, exposure to toluene, and sham exposure. The peak blood concentration of solvent was known to be reached at the end of the exposure, based on previous studies.[22] Testosterone concentrations were also measured in three plasma samples from the end of the period before exposure, the end of the exposure period, and the end of the period after exposure in the men only.

EXPOSURE SYSTEM

Subjects breathed air containing 50 ppm toluene for 180 minutes. This is equivalent to 18.75 ppm 8 hour time weighted average (TWA) or 37.5% of the American Conference of Government Industrial Hygienists (ACGIH) maximum recommended daily exposure of 50 ppm 8 hour TWA[23]; and 18.8% of the United States Occupational Safety and Health Administration (OSHA) permissible exposure limit. This level of exposure was chosen because effects on gonadotropin secretion have been reported in workers exposed to similar concentrations,[7 8] because most current occupational exposure limits range between 50 and 100 ppm, 8 hour TWA,[23] and because it is a level which occurs in many workplaces.[24]

The exposure system consists of a dynamic atmosphere generator, a gated inhalation port, and sensors for monitoring toluene concentration.[25] Briefly, a solvent syringe pump (Harvard apparatus infusion/withdrawal pump model 920; Holliston, MA, USA) delivers a constant flow rate of solvent to a heater where it is vaporised; solvent vapour is diluted with filtered air in a glass mixing chamber, and from there the air-toluene mixture flows to a mouthpiece. Subjects with nose clips inhale the mixture in a seated position. Two one way valves in the mouthpiece prevent reinhalation of exhaled air. During the exposure period the inhaled toluene concentration is continuously monitored by photoionisation detector.

ASSAY OF BIOLOGICAL SAMPLES

Hormone assays

Plasma LH and FSH were measured with Wallac DELFIA time resolved fluoroimmunoassays (Turku, Finland) by a blinded technician. Each assay uses two monoclonal antibodies (mouse anti-human) directed against two different antigenic determinants of the gonadotropin. For the present study each subject sample was run in duplicate, and the mean of the two concentrations was used for statistical calculations and graphical presentation of the data. Serum control pools of known low, medium, and high concentrations were run in quadruplicate with each assay. For the present studies the coefficients of variation (CVs) between assays for LH ranged from 9.0% to 11.6% for the different control pools, and for FSH the CVs between assays ranged from 6.0% to 21.8%, which are typical ranges for gonadotropin immunoassays.[15 20] Plasma testosterone was measured by radioimmunoassay with reagents from the World Health Organisation match reagent programme by previously described methods.[26] Between assay CVs ranged from 14% to 24% for the different control serum pools.

Toluene assay

Blood drawn during the exposure was analysed for toluene with a modification of the static headspace/on column cryofocusing analysis method of Dills *et al*.[27] Toluene contamination of the heparinised Vacutainer tubes has been found to be negligible in comparison with the blood concentrations reached during and for several hours after a 3 hour, 50 ppm exposure.

PULSE ANALYSIS

Pulses of LH and FSH were identified with the ULTRA pulse detection program.[28] This program takes as input the sample hormone concentrations, the CVs for the concentration ranges of the assay, and a threshold expressed in terms of a multiple of assay CVs. The program proceeds by eliminating all increments and decrements from the series that do not exceed the specified multiple of local assay CVs. The remaining peaks are considered to be relevant pulses. Previous studies have used thresholds of 2× to 4× assay CVs[15 18] or 2× assay standard deviation[16 17 20] to optimise detection of actual pulses while minimising false positives. Based on initial comparisons of 2× and 3× CV thresholds, a 3× CV threshold was chosen for this study to minimise false positive pulses.

STATISTICAL ANALYSIS

The study analysis used a repeated measures analysis of variance (ANOVA) design. In this design the dependent variables (mean concentrations, pulse amplitude, and pulse frequency of LH and FSH) are measured repeatedly for each subject. Therefore, as well as the variable "exposure" between subjects (toluene or sham), there is also a variable "sampling period" within subjects (before, during, and after exposure), and the variable of most interest is the interaction term between exposure and sampling period.

Three female subjects were excluded from the final analyses because their LH and FSH profiles were not consistent with the expected stage of the menstrual cycle: one woman in the follicular phase with sham exposure; one woman in the luteal phase exposed to toluene; and one woman in the luteal phase with sham exposure. Pulse amplitude, pulse frequency, and mean hormone concentrations for the remaining subjects were assessed from the results of the ULTRA analysis and were compared before, during, and after exposure with repeated measures ANOVA and analysis of covariance (ANCOVA). The ANCOVAs were performed with age, body mass index (BMI, calculated as weight (kg)/height (m)^2), and adipose tissue fraction assessed by skin fold thickness[29] as covariates. These covariates were chosen because gonadotropin concentrations in both sexes increase with age[30 31]; obesity is associated with changes in gonadotropin concentrations,[32 34] and toluene body burdens are influenced by body composition.[22] Differences were considered significant if the p value was ≤ 0.05.

When data for a particular variable were not normally distributed, a log transformation was used. If the transformed data were still not normally distributed (as was the case for LH and FSH pulse frequency in all of the groups), the Kruskal-Wallis test for non-parametric data was used to compare the sham group with the group exposed to toluene during the exposure and after the exposure.

Results

DEMOGRAPHIC INFORMATION AND PHYSICAL CHARACTERISTICS OF STUDY PARTICIPANTS

Age, height, weight, BMI, and adipose tissue fraction for the study groups are summarised in

Table 1 *Characteristics of participants*

	Exposure to toluene mean (range)	*Sham exposure mean (range)*	*p Value*
Women in the follicular phase:			
n	5	4	
Age (y)	27.4 (22–31)	24.5 (20–26)	0.24
Weight (kg)	60.9 (48.5–67.4)	93.0 (59.5–127.5)	0.13
Height (cm)	163.5 (150–168)	166.5 (162.0–170.5)	0.46
BMI	22.7 (21.4–24.2)	33.6 (20.5–45.4)	0.15
Adipose tissue (%)	23.3 (21.3–26.5)	29.1 (20.7–34.3)	0.17
Day of study*	6.6 (5 to 8)	6.4 (5 to 8)	0.83
Women in the luteal phase:			
n	5	4	
Age (y)	25.8 (19–36)	24.8 (20–33)	0.81
Weight (kg)	67.8 (55.1–92.0)	70.0 (58.0–89.0)	0.83
Height (cm)	162.7 (157–175.5)	170.8 (162.0–177.0)	0.12
BMI	25.5 (22.2–34.6)	24.0 (19.8–28.4)	0.59
Adipose tissue (%)	25.4 (21.9–28.7)	22.6 (20.8–26.0)	0.12
Day of study†	−6.6 (−5 to −9)	−6.5 (−5 to −8)	0.93
Men:			
n	5	5	
Age (y)	30.2 (23–44)	32.8 (23–39)	0.59
Weight (kg)	82.7 (73.5–92)	78.1 (70–85.5)	0.30
Height (cm)	180.3 (177–187)	176.8 (167–182)	0.32
BMI	25.5 (22.2–29.0)	25.0 (24.2–25.8)	0.69
Adipose tissue (%)	18.9 (16.5–23.4)	19.4 (15.7–23.9)	0.88

*Relative to first day of present menstrual cycle.
†Relative to expected first day of next menstrual cycle.

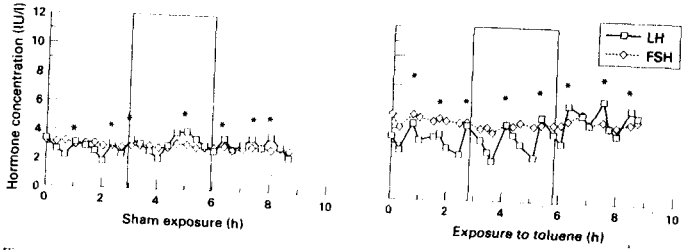

Figure 1 LH and FSH secretion profiles in representative, individual women in the follicular phase exposed and not exposed to toluene. Blood samples, indicated by the open symbols, were drawn every 20 minutes for 9 hours. Exposure interval is indicated by the rectangle. Significance for LH and FSH^ pulses were assessed by the ULTRA program[20] with a difference of 3× local assay coefficients of variation from trough to peak as the criterion.*

table 1. There were no significant differences between the sham group and the group exposed to toluene, despite the presence of one woman in the follicular phase with sham exposure who had a high BMI and adipose tissue fraction. Moreover, the results of this and subsequent analyses did not substantially change when data from this subject were removed.

SYMPTOMS RELATED TO EXPOSURE

None of the subjects complained of any headaches, lassitude, or loss of appetite, which have been published in association with exposures to toluene of 50–200 ppm.[21] Both groups of subjects complained of mild discomfort related to sitting relatively still for 3 hours and of a dry mouth.

BLOOD TOLUENE CONCENTRATIONS IN MEN AND WOMEN AFTER CONTROLLED EXPOSURE

Inhalational exposure to 50 ppm toluene for 3 hours resulted in mean (SEM) peak blood toluene concentrations of 4.90 (0.97) nmol/ml (range 3.58–6.79) in men; 3.65 (1.02) nmol/ml in women during the luteal phase (range 0.23–5.72); and 5.57 (1.09) nmol/ml in women during the follicular phase (range 2.32–7.96). One outlier was identified: a woman in the luteal phase with exposure to toluene. Analyses performed without this outlier did not substantially differ from analyses with the outlier included, so the outlier was included in the results. Mean toluene concentrations in subjects with sham exposure also did not differ by sex or cycle stage (0.013 (0.004) nmol/ml in men, 0.010 (0.005) nmol/ml in luteal women, 0.011 (0.006) nmol/ml in follicular women).

EFFECT OF EXPOSURE TO TOLUENE ON LH AND FSH IN WOMEN DURING FOLLICULAR AND LUTEAL PHASES

Figure 1 depicts representative individual LH and FSH profiles before, during, and after sham exposure and exposure to toluene, respectively, in women during the follicular phase. High frequency, low amplitude LH pulses and infrequent, low amplitude FSH pulses occurred in both groups of women studied during days 5–9 of their menstrual cycles.

ANCOVA with age, BMI, or adipose tissue fraction as the covariate showed no significant effect of these possible covariates on LH or FSH secretion. Therefore, only the results of the unadjusted analyses will be reported here. The mean LH and FSH concentrations, pulse amplitudes, and pulse frequencies are depicted in figure 2 for women in the follicular phase. There was no significant effect of exposure to toluene alone, nor were there significant interactions between exposure and sampling period, on LH mean concentration, pulse amplitude or pulse frequency, or on FSH mean or log FSH mean concentrations in women in the follicular phase. There was a significant effect of sampling period alone ($p=0.04$) on LH mean concentration and log LH mean concentration, in the form of slightly increasing mean LH concentrations in both groups of women over the three sampling periods. Few significant FSH pulses were found in the group exposed to toluene and no FSH pulses were found in the group with sham exposure. Therefore, analyses were not done for FSH pulse amplitude or pulse frequency in women in the follicular phase.

Figure 3 depicts representative individual LH and FSH profiles in women in the luteal phase. In these women studied during days 19–23 of their cycles low frequency, high amplitude LH pulses and infrequent, small amplitude FSH pulses were found.

The mean LH and FSH concentrations, pulse amplitudes, and pulse frequencies are depicted in figure 4 for women in the luteal phase. There was no significant effect of exposure to toluene in these women, nor were there significant interactions between exposure and sampling period, on LH mean concentrations, pulse amplitude, or pulse frequency. However, there was a trend towards a significant interaction ($p=0.06$) between sampling period and exposure on LH pulse frequency, with pulse frequency apparently reduced during the exposure period in the women with exposure to toluene compared with the sham exposure. There was a significant effect of sampling period alone on mean concentrations ($p=0.02$) and pulse frequency of LH ($p=0.04$), reflecting the occurrence of most of the luteal phase pulses during the intervals before and after exposure in both exposure groups. There was no significant effect of exposure, nor was there interaction between exposure and sampling

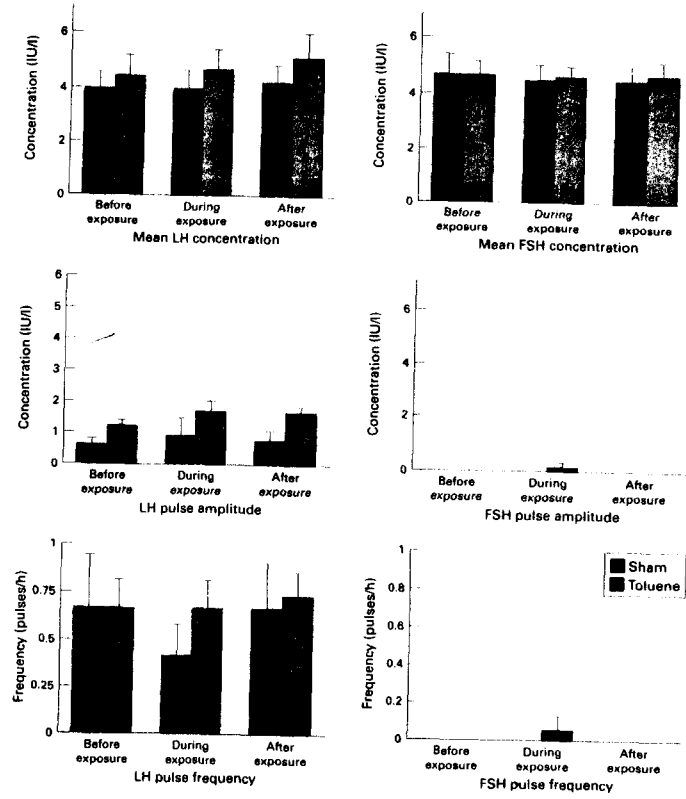

Figure 2 LH and FSH secretion in women in the follicular phase with sham exposure and exposure to toluene. Bars show mean (SEM) for each 3 hour sampling period for groups exposed (n = 5) or not exposed (n = 4) to toluene, $p<0.05$, ANOVA, effect of sampling period on LH mean concentration.

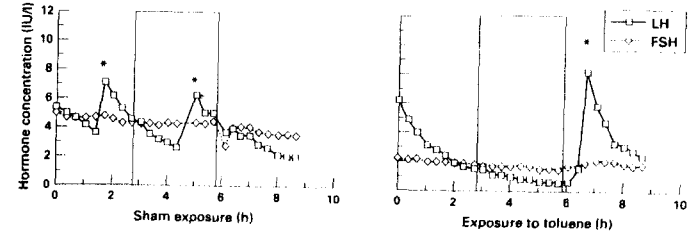

Figure 3 LH and FSH secretion profiles in representative women in the luteal phase exposed or not exposed to toluene. Exposure interval is indicated by the rectangle. Significant LH and FSH^ pulses are indicated. Further details in legend to figure 1.*

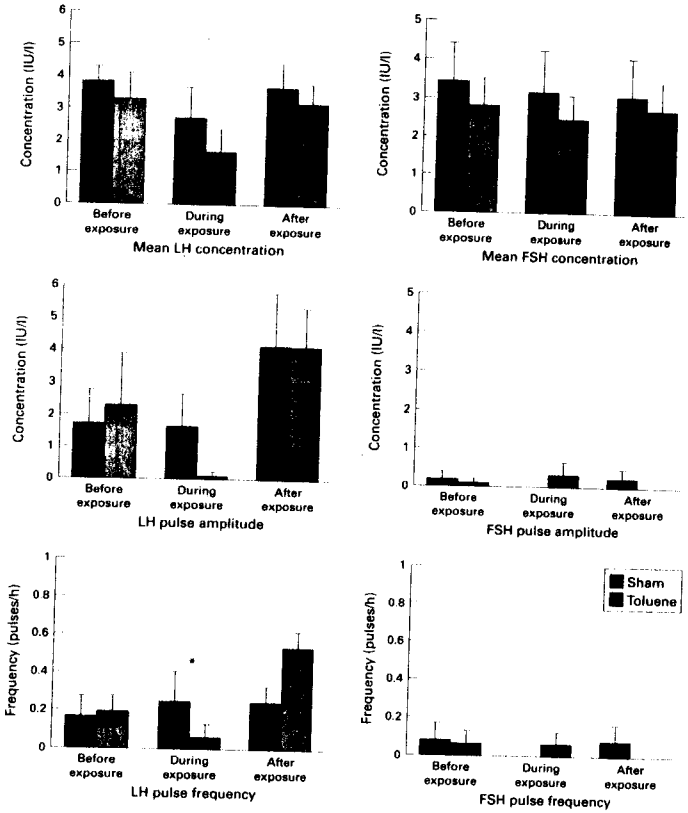

*Figure 4 LH and FSH secretion in women in the luteal phase exposed or not exposed to toluene. Bars show mean (SEM) for each 3 hour sampling period for groups with sham exposure (n=4) and exposure to toluene (n=5). * p=0.06 for the interaction between exposure and sampling period on LH pulse frequency. p<0.05 by ANOVA for the effect of sampling period on LH mean concentration and pulse frequency and on FSH mean concentration.*

period on mean FSH concentrations; however, there was a borderline significant effect of sampling period alone (p=0.05), reflecting slightly higher mean FSH concentrations before exposure than during and after exposure in both sham and exposed groups. There were no significant effects on FSH pulse amplitude or frequency.

EFFECT OF EXPOSURE TO TOLUENE ON LH AND FSH IN MEN

Figure 5 depicts representative individual LH and FSH profiles before, during, and after sham exposure and exposure to toluene in men. In both exposure groups of men the LH pulses consisted of low amplitude fluctuations occurring at frequencies varying among men from about 1/90 minutes to 1/3 hours. In comparison FSH fluctuations were small and infrequent in both groups.

Analyses by ANCOVA performed with age, BMI, or adipose tissue fraction as the covariate showed a significant effect of age on LH pulse frequency, but no other significant effects of these covariates on LH or FSH secretion, nor on testosterone secretion. There was no significant effect of treatment or significant interaction between treatment and sampling period either when the LH pulse frequency data were analysed by ANOVA or by ANCOVA with age as a covariate. Therefore, the results of the unadjusted ANOVAs are reported here. The mean LH and FSH concentrations, pulse amplitudes, and pulse frequencies in men with sham exposure and exposure to toluene are depicted in figure 6. There was no significant effect of treatment alone on any variable of LH secretion; however, there was a significant interaction (p<0.05) between treatment and sampling period on LH mean concentration.

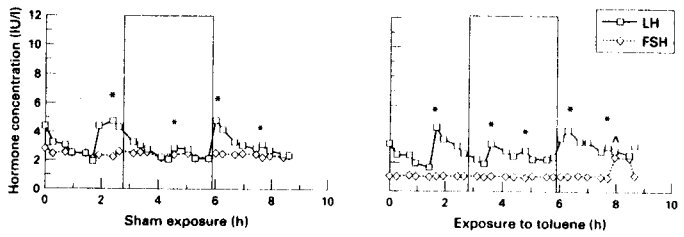

*Figure 5 LH and FSH secretion profiles in representative men with sham exposure and exposure to toluene. Exposure interval is indicated by the rectangle. Significant LH * and FSH ^ pulses are indicated. Further details from legend to figure 1.*

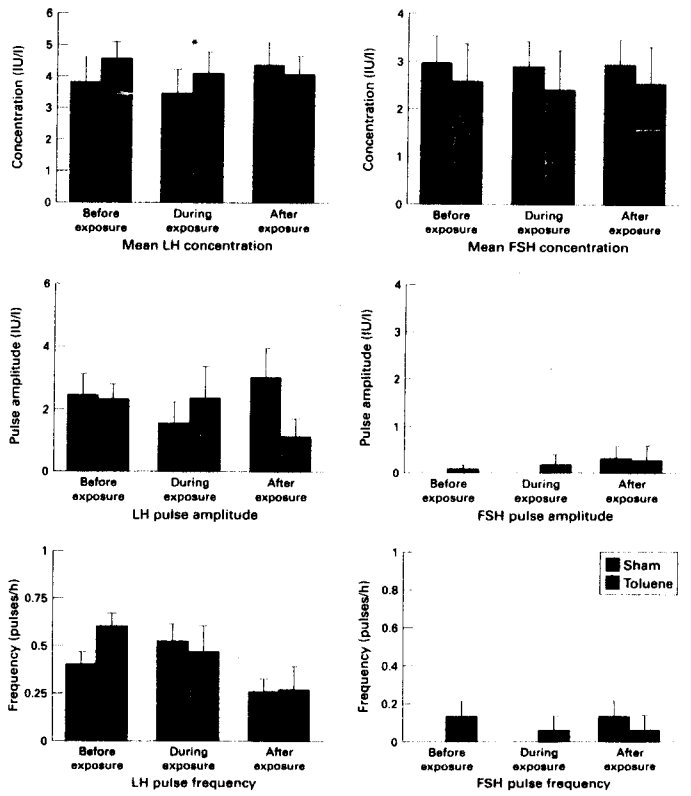

*Figure 6 LH and FSH secretion in men with sham exposure and exposure to toluene. Bars show mean (SEM) for each of the 3 hour sampling periods for n=5 subjects/group. *p<0.05 by ANOVA for the interaction between sampling period and exposure on LH mean concentration.*

Table 2 Mean (SEM, range) testosterone concentrations in men

Sampling period	*Sham exposure (nmol/l) n=5*	*Exposure to toluene (nmol/l) n=5*
Before exposure	17.5 (2.1, 11.6 20.6)	19.6 (1.1, 16.3 22.2)
During exposure	17.0 (2.2, 13.3 23.3)	22.9 (2.8, 17.2 29.8)
After exposure	16.9 (0.7, 14.3 18.1)	19.8 (1.9, 13.7 25.3)

There were no significant differences among groups or time intervals by ANOVA.

This was in the form of slightly declining mean LH concentrations over the exposure intervals in the men exposed to toluene, whereas LH concentrations decreased and then increased slightly during and after sham exposure. There were no significant effects of treatment or exposure interval on FSH mean concentration, log FSH mean concentration, pulse amplitude, or pulse frequency in men.

Plasma testosterone concentrations measured at the end of each of the three time intervals did not differ by exposure or sampling period (table 2).

Discussion

The present study was designed to test the hypothesis that exposure to toluene at 50 ppm for 3 hours acutely suppresses LH and FSH secretion through suppression of hypothalamic GnRH pulse frequency in men and women. This exposure is equivalent to 38% of the ACGIH threshold limit value TWA, 38% of the British regulatory exposure limit, and 19% of the United States and German regulatory exposure limits.[23] Exposure to toluene at this concentration did not result in abnormal LH or FSH pulse amplitude, pulse frequency, or mean concentrations in either men or women, as defined by previous studies of spontaneous episodic LH and FSH secretion in young men[15 17] and in women in either the luteal or follicular phase.[16 18 20 35] Of interest, however, was (*a*) a trend towards a significant decline in LH pulse frequency in women in the luteal phase during exposure to toluene (p=0.06), and (*b*) a small, but significant, decline in LH mean concentrations in men exposed to toluene compared with the sham group.

The clinical relevance of the observed effects is unclear. The mean LH concentrations in all of the men remained within the normal ranges for LH mean concentrations which were established by the laboratory which performed the assays (personal communication). Therefore, the observed effect on mean LH concentrations may represent a subclinical effect of toluene on the pituitary or hypothalamus without physiological impact on testicular testosterone secretion. The trend towards decreased LH pulse frequency in women during the luteal phase exposed to toluene is also difficult to interpret. It may be coincidental as LH pulse amplitude and mean concentration also declined during sham exposure, although not to the same degree as during exposure to toluene. Although the very low LH pulse frequency found in the women exposed to toluene during the 3 hour exposure period falls outside of the normal range of about 0.2–0.5 pulses/hour during a 24 hour period in the mid-luteal phase, periods of 4–5 hours in duration with no notable LH pulses occur commonly in women during this phase.[16 18 20 35] For comparison LH pulse frequencies of 0–0.25 pulses/hour have been reported in women with hypothalamic amenorrhoea or oligomenorrhoea.[36 37] Because of the low LH pulse frequency in normal women during the luteal phase, longer exposures with frequent blood sampling may be necessary to find whether exposure to toluene suppresses LH pulse frequency in women during the luteal phase.

As each peripheral LH pulse is a reflection of a hypothalamic GnRH pulse,[19] the absence of an effect of exposure to toluene on LH pulse frequency suggests that intermittent acute exposure to toluene at concentrations up to 50 ppm probably does not acutely alter GnRH pulse frequency. Our results seem to contradict those of two epidemiological studies which found inverse associations between mean concentrations of LH, FSH, and testosterone and personal breathing zone toluene concentrations below the current threshold limit value.[7 8] The peak blood toluene concentrations found in this study after 3 hours inhalation of 50 ppm toluene (0.23 7.96 nmol/ml) were of the same order of magnitude as the blood toluene concentrations measured in those studies, suggesting that lower mean exposure concentrations in our study were not the cause of the difference. Several other factors may explain the disparities. Firstly, the workers in those studies were exposed to toluene on a daily basis for years. Although the authors found a significant association only with current toluene concentrations and not with lifetime exposure, it is possible that a longer period of exposure to toluene is required for effects on gonadotropin secretion to occur. Secondly, by contrast, Mørck and coworkers found cumulative lifetime exposure to toluene to be positively correlated with concentrations of plasma FSH, but not with LH or testosterone.[38] The discrepancies among these previous studies, which used single blood samples for gonadotropin measurements, and our study may also be due to the different sampling regimens used. Our technique is expected to provide much more precise estimates of mean hormone concentrations, as well as providing information about pulse amplitude and frequency. Thirdly, the workers in the studies of Svensson and coworkers were subject to fluctuating toluene concentrations, rather than constant concentrations as in our experiments. Although the median TWAs were 36 ppm[7] and 47 ppm,[8] concentrations as high as 111 and 142 ppm, respectively, were found. It is possible that the peak concentration of exposure to toluene rather than TWA exposure is the important factor affecting gonadotropin secretion. Finally, the subjects in our study were exposed to toluene vapour at rest, whereas printing workers are engaged in physical activity while performing their work tasks. The increasing minute ventilation which occurs with increasing exertion results in greater absorbed doses of toluene for a given exposure.[39] However, the measured whole blood concentrations of toluene in the studies of Svensson *et al*[7 8] were of the same order of magnitude as the peak exposures in our study.

There are several important limitations to our study. Firstly, it is possible that the sample size was insufficient to detect a small effect of toluene on LH and FSH secretion. However, these studies had statistical power of at least 80% to detect a 12% change in mean LH concentrations and a 4% change in mean FSH concentrations in all three groups, and a 20%, 30%, and 100% change in mean LH pulse amplitude in women in the follicular phase, the luteal phase, and men, respectively. Changes of these magnitudes would be unlikely to push LH and FSH secretion out of the normal ranges reported[15 17 18 20] and would thus represent subclinical effects. Secondly, the present results cannot rule out the possibility that exposures to higher concentrations of toluene or longer exposure periods would result in significant changes in gonadotropin secretion in men or women. Previous studies, however, have shown that other effects of toluene on the CNS can be found after <3 hours exposure. For example, controlled exposure studies in humans have found that visual vigilance task performance was impaired after inhaling 100 ppm for 2 hours and that reaction times were increased after 20 minutes inhalational exposures to 300 ppm.[23] ATPase activities in primary astrocyte cultures[40] and in synaptosomal membrane preparations[41] were inhibited after 1 hour and 40 minutes exposure to toluene, respectively. Although no studies have been published describing effects of acute exposure to toluene on the catecholaminergic neurons in the hypothalamus involved in regulation of GnRH secretion, numerous studies have shown that GnRH secretion can be acutely altered by the administration of various pharmacological agents—such as the excitatory amino acid N-methyl-D,L-aspartate or the opiate antagonist naloxone.[42 43] Subacutely, exposure to toluene increases noradrenaline concentrations and turnover in the hypothalamus.[41] It may be that toluene induces such changes by mechanisms which do not operate acutely—such as alterations in the transcription of genes encoding enzymes in the noradrenaline synthetic or metabolic pathways. It is also possible that effects on LH and FSH secretion which have been noted by others were not mediated by hypothalamic actions of toluene, but rather by effects on the pituitary which do not occur acutely—such as alteration of gonadotropin subunit transcription or translation.

Another possible explanation for the discrepancy between previously published studies of the effect of workplace exposure to toluene on gonadotropin concentrations and the present study is confounding. We excluded subjects with conditions which might affect gonadotropin concentrations such as chronic illnesses, infertility, smoking, problem drinking, and age >45. We also used a randomised study design to minimise confounding by other unanticipated factors and used ANCOVA to control for covariates. Previous studies had controlled for age[8] and alcohol use,[7] but not for smoking. Smoking is known to result in lowered sperm density and increased testosterone concentrations, but has not been found to affect LH and FSH concentrations.[44] Therefore, smoking is not likely to have been a significant confounder in previous studies. We also evaluated the potential covariates age, BMI, and adipose tissue fraction and found no significant effects on variables of LH or FSH secretion. The absence of an effect of age in our young subjects (only one >40) is not surprising. Previous studies have found that LH and FSH concentrations do not begin to increase significantly in women or men until ages >40.[30 31 45] The relation between adiposity and reproductive endocrine function seems to be more complicated, with conflicting effects of obesity on gonadotropin secretion reported for both men[13 46] and women.[32 34] Various criteria for obesity were used in these studies, but the BMIs generally were >30. As we had no male subjects with BMIs >30 and only three women with BMIs in this range, it is not surprising that we did not find significant associations between measures of adiposity and gonadotropin secretion.

In summary, exposure of men and normally cycling women of reproductive age to 50 ppm toluene for 3 hours at rest did not result in abnormal patterns of episodic LH or FSH secretion. We did find a possible LH pulse frequency change in women during the luteal phase and subtle changes in mean concentration of LH in men exposed to toluene. As peripheral pulses of LH are preceded by hypothalamic GnRH pulses, these results also provide evidence that acute exposure to these concentrations of toluene are unlikely to affect GnRH pulse frequency in men or women in the follicular phase. The results further suggest that intermittent, acute exposures to concentrations up to 50 ppm toluene, which are separated by sufficient time for toluene to be cleared from the body, are unlikely to result in physiologically important changes in gonadotropin secretion. This study cannot, however, rule out the possibility that longer durations of exposure or exposure during periods of physical exertion to comparable concentrations of toluene may have greater effects on gonadotropin secretion. Our findings indicate the need for further studies of reproductive function in workers exposed chronically to toluene to establish the safety of present occupational exposure limits.

We thank Dorothy McGuinness and Elizabeth Van Gaver of the Population Center for Research in Reproduction at the VA Puget Sound Health Care Center for their excellent technical help in performing the gonadotropin and testosterone assays and Mike Wessels for his technical expertise in performing the toluene assays. We thank Dr William Bremner, director of the Population Center, for his support and feedback on this study. We are also grateful to Dr Lianne Shepard for advice on the statistical analyses. Funding for this project was provided by NIOSH grant (to UL) 1 R03 OH03468-01; by the Consortium for Risk Evaluation with Stakeholder Participation, US DOE Cooperative Agreement with the University of Washington DE-FC01-95EW55084; and by a University of Washington NIEHS Center for Ecogenetics and Environmental Health (1 P30 ES07033-03) small grant (to UL). Additional support was provided by the Workers' Compensation Fund of Washington State, which is administered by the Washington Department of Labor and Industries. Portions of this work were presented at the 8th International Congress of Toxicology, Paris, France, 5–9 July 1998.

1 Barlow SM, Sullivan FM. *Reproductive hazards of industrial chemicals*. New York: Academic Press, 1982.
2 Huang XY. Influence on benzene and toluene to reproductive function of female workers in leathershoe-making industry [abstract]. *Chinese Journal of Preventive Medicine* 1991;25:89–91.
3 Sallmen M, Lindbohm M-L, Kyyronen P, *et al*. Reduced fertility among women exposed to organic solvents. *Am J Ind Med* 1995;27:699–713.
4 Sallmén M, Lindbohm M-L, Anttila A, *et al*. Time to pregnancy among the wives of men exposed to organic solvents. *Occup Environ Med* 1998;55:24–30.
5 Smith EM, Hammonds-Ehlers M, Clark MK, *et al*. Occupational exposures and risk of female infertility. *J Occup Environ Med* 1997;39:138–47.
6 Ng TP, Foo SC, Yoong T. Menstrual function in workers exposed to toluene. *Br J Ind Med* 1992;49:799–803.
7 Svensson B-G, Nise G, Erfurth EM, *et al*. Neuroendocrine effects in printing workers exposed to toluene. *Br J Ind Med* 1992;49:402–8.
8 Svensson B-G, Nise G, Erfurth E-M, *et al*. Hormone status in occupational toluene exposure. *Am J Ind Med* 1992;22:99–107.
9 Ameno K, Kiriu T, Fuke C, *et al*. Regional brain distribution of toluene in rats and in a human autopsy. *Arch Toxicol* 1992;66:153–6.
10 Stepanov MG, Altukhov VV, Proumina FI, *et al*. Physiologic mechanisms of the reaction of the reproductive system in female rats to chronic exposure to low doses of toluene [abstract]. *Fiziologicheskii Zhurnal SSSR Imeni IM Sechenova* 1990;76:1096–102.
11 Andersson K, Fuxe K, Tottgard R, *et al*. Toluene-induced activation of certain hypothalamic and median eminence catecholamine nerve terminal systems of the male rat and its effects on anterior pituitary hormone secretion. *Toxicol Lett* 1980;5:393–8.
12 von Euler G, Fuxe K, Hansson T, *et al*. Effects of chronic toluene exposure on central monoamine and peptide receptors and their interactions in the adult male rat. *Toxicology* 1988;52:103–26.
13 Wemer RI, Findell PR, Kordon C. Role of classic and peptide neuromediators in the neuroendocrine regulation of LH and prolactin. In: Knobil E, Neill JD, eds. *The physiology of reproduction, 2nd ed*. New York: Raven Press, 1994:1235–81.
14 Santen RJ, Bardin CW. Episodic luteinizing hormone secretion in man: pulse analysis, clinical interpretation, physiologic mechanisms. *J Clin Invest* 1973;52:2617–28.
15 Matsumoto AM, Bremner WJ. Modulation of pulsatile gonadotropin secretion by testosterone in man. *J Clin Endocrinol Metab* 1984;58:609–13.
16 Soules MR, Steiner RA, Cohen NL, *et al*. Nocturnal slowing of pulsatile luteinizing hormone secretion in women during the follicular phase of the menstrual cycle. *J Clin Endocrinol Metab* 1985;61:43–9.
17 Spratt DI, O'Dea LSL, Schoenfeld D, *et al*. Neuroendocrine-gonadal axis in men: frequent sampling of LH, FSH, and testosterone. *Am J Physiol* 1988;254:E658–66.
18 Filicori M, Santoro N, Merriam GR, *et al*. Characterization of the physiological pattern of episodic gonadotropin secretion throughout the human menstrual cycle. *J Clin Endocrinol Metab* 1986;62:1136–44.
19 Plant TM. Gonadal regulation of hypothalamic gonadotropin-releasing hormone release in primates. *Endocr Rev* 1986;7:75–88.
20 Soules MR, Steiner RA, Clifton DK, *et al*. Progesterone modulation of pulsatile luteinizing hormone secretion in normal women. *J Clin Endocrinol Metab* 1984;58:378–83.
21 Hotchkiss J, Knobil E. The menstrual cycle and its neuroendocrine control. In: Knobil E, Neill JD, eds. *The physiology of reproduction, 2nd ed*. New York: Raven Press, 1994:711–49.
22 Pierce C, Dills R, Morgan M, *et al*. Interindividual differences in 2H8-toluene toxicokinetics assessed by a semi-empirical physiologically-based model. *Toxicol Appl Pharmacol* 1996;139:49–61.
23 American Conference of Governmental Industrial Hygienists. *Documentation of the threshold limit values and biological exposure indices*, 6th ed. Cincinnati, OH: ACGIH, 1991.
24 Agency for Toxic Substances and Disease Registry. *Toxicological profile for toluene (update)* Atlanta: US Department of Health and Human Services, Public Health Service, ATSDR, 1994. (ATSDR/TP-93/14 ed.)
25 Morgan M, Dills RL, Kalman DA. Evaluation of stable isotope-labeled probes in the study of solvent pharmacokinetics in human subjects. *Int Arch Occup Environ Health* 1993;651:S139–42.
26 Matsumoto AM, Paulsen CA, Hopper BR, *et al*. Human chorionic gonadotropin and testicular function: stimulation of testosterone, testosterone precursors, and sperm production despite high estradiol levels. *J Clin Endocrinol Metab* 1983;56:720–8.
27 Dills RL, Kent SD, Checkoway H, *et al*. Quantification of volatile solvents in blood by static headspace analysis. *Talanta* 1991;38:365–74.
28 Van Cauter E. Estimating false-positive and false-negative errors in analyses of hormonal pulsatility. *Am J Physiol* 1988;254:E786–94.
29 Durnin JVGA, Womersly J. Body fat assessed from total body density and its estimation from skin fold thickness: measurements on 481 men and women aged from 16 to 72 years. *Toxicol Appl Pharmacol* 1974;120:266–73.
30 Klein NA, Battaglia DE, Clifton DK, *et al*. The gonadotropin secretion pattern in normal women of advanced reproductive age in relation to the monotropic FSH rise. *Journal of the Society for Gynecologic Investigations* 1996;3:27–32.
31 Deslypere JP, Vermeulen A. Leydig cell function in normal men: effect of age, life style, residence, diet, and activity. *J Clin Endocrinol Metab* 1984;59:955–62.
32 Grenman S, Ronnemaa T, Irjala K, *et al*. Sex steroid, gonadotropin, cortisol, and prolactin levels in healthy, massively obese women: correlation with abdominal fat cell size and effect of weight reduction. *J Clin Endocrinol Metab* 1986;63:1257–61.
33 Vermeulen A, Kaufman JM, Deslypere JP, *et al*. Attenuated luteinizing hormone (LH) pulse amplitude but normal LH pulse frequency, and its relation to plasma androgens in hypogonadism of obese men. *J Clin Endocrinol Metab* 1993;76:1140–6.
34 Unzer SRM, dos Santos JE, Moreira AC, *et al*. Alterations in plasma gonadotropin and sex steroid levels in obese ovulatory and chronically anovulatory women. *J Reprod Med* 1995;40:516–20.
35 Rossmanith WG, Liu CH, Laughlin GA, *et al*. Relative changes in LH pulsatility during the menstrual cycle: using data from hypogonadal women as a reference point. *Clin Endocrinol* 1990;32:647–60.
36 Veldhuis JD, Evans WS, Demers LM, *et al*. Altered neuroendocrine regulation of gonadotropin secretion in women distance runners. *J Clin Endocrinol Metab* 1985;61:557–63.
37 Southworth MB, Matsumoto AM, Gross KM, *et al*. The importance of signal pattern in the transmission of endocrine information: pituitary gonadotropin responses to continuous and pulsatile gonadotropin-releasing hormone. *J Clin Endcrinol Metab* 1991;72:1286–9.
38 Mørck HI, Winkel P, Gyntelberg F. Health effects of toluene exposure. *Dan Med Bull* 1988;35:196–200.
39 Mur TF, Pierce CH, Morgan MS, *et al*. The effects of exercise and gender on toluene toxicokinetics in human volunteers [abstract]. *Toxicol Sci* 1998;42(suppl 1):142.
40 Vaalavirta L, Tähti H. Effects of selected organic solvents on the astrocyte membrane ATPase in vitro. *Clin Exp Pharmacol Physiol* 1995;22:293–94.
41 Edeltors S, Ravn-Jonsen A. Effects of simultaneous ethanol and toluene exposure on nerve cells measured by changes in synaptosomal calcium uptake and (Ca^{2+}/Mg^{2+})-ATPase activity. *Pharmacol Toxicol* 1991;69:90–5.
42 Gay VL, Plant TM. N-methyl-D,L-aspartate elicits hypothalamic gonadotropin-releasing hormone release in prepubertal male rhesus monkeys (Macaca Mulatta). *Endocrinology* 1987;120:2289–96.
43 Karahalios DG, Levine JE. Naloxone stimulation of in vivo LHRH release is not diminished following ovariectomy. *Neuroendocrinology* 1988;47:504–10.
44 Vogt H-J, Heller W-D, Burelli S. Sperm quality of healthy smokers, ex-smokers, and never-smokers. *Fertil Steril* 1986;45:106–10.
45 Schmidt PJ, Gindoff PR, Baron DA, *et al*. Basal and stimulated gonadotropin levels in the perimenopause. *Am J Obstet Gynecol* 1996;175:643–50.
46 Blank DM, Clark RV, Heymsfield SB, *et al*. Endogenous opioids and hypogonadism in human obesity. *Brain Res Bull* 1994;34:571–4.

Mortalities of workers at the Nitro plant with exposure to 2-mercaptobenzothialzole

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Abstract

***Objectives*—An update of a study of workers exposed to 2-mercaptobenzothiazole (MBT) at a rubber chemicals plant in Nitro, West Virginia is reported. The earlier study found high rates of lung cancer, prostate cancer, and bladder cancer in these workers who also had potential exposure to 4-aminobiphenyl (PAB), a potent bladder carcinogen.**

***Methods*—This cohort mortality study examines the mortalities of 1059 full time white male production workers employed at the plant from 1955 to 1977. A detailed exposure assessment was done on the 600 workers with exposure to MBT. Nine years of additional follow up to the previous study are added.**

***Results*—It was found that MBT workers have expected rates of lung (standardised mortality ratio (SMR)=1.0 95% confidence interval (95% CI) 0.7 to 1.5) and prostate (SMR=0.9, 95% CI 0.2 to 2.3) cancer. There was an excess of bladder cancer among MBT workers who had definite exposure to PAB (SMR=27.1, 95% CI 11.7 to 53.4), and MBT workers with potential exposure to PAB (SMR=4.3, 95% CI 1.4 to 10.0). However, there were no deaths from bladder cancer among workers with no exposure to PAB (SMR=0.0, 95% CI 0.0 to 24.7), although there were only 0.2 deaths expected.**

***Conclusions*—The potential confounding of exposure to an unknown portion of PAB in the MBT workers makes it impossible to evaluate risk of bladder cancer in this population at this time. However, exposure to MBT does not seem to increase the risk of most cancers including cancers of the lung and prostate.**

(*Occup Environ Med* 1999;56:667–671)

Keywords: mercaptobenzothiazole; 4-aminobiphenyl; cancer; confounding exposure; exposure misclassification

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Accepted 17 May 1999

2-Mercaptobenzothiazole (CAS No 149-30-4) (MBT) is a rubber chemical accelerator which improves the vulcanisation process. Vulcanisation is a procedure that stabilises rubber by creating crosslinks between polymers to provide strength and elasticity. There is some evidence of carcinogenic activity of MBT in one animal species.[1] Rats developed dose related tumours of the adrenal gland but there is no carcinogenic activity in mice.[1]

Two epidemiology studies reported on the cancer rates of workers with exposure to MBT at rubber chemicals plants.[2 3] A study of MBT workers in Ruabon, Wales reported that rates of most cancers and other causes of death were lower than or equal to local population rates.[2] This study, however, reported increased rates of bladder cancer, but many of these workers also had exposure to the suspect bladder carcinogen o-toluidine, phenyl-β-naphthylamine, which may contain β-naphthylamine, a bladder carcinogen, and MBT.[2] Another study of MBT workers at a plant in Nitro, West Virginia reported high rates of bladder, lung, and prostate cancer. These high rates of cancers were limited to MBT workers with potential exposure to p-aminobiphenyl (PAB), a potent bladder carcinogen.[3] However, the MBT workers without exposure to PAB had no bladder, lung, or prostate cancers, although few were expected.

The potential confounding from carcinogens in both these studies has made interpretation of the cancer findings for MBT workers difficult. Further follow up provides more precise risk estimates and more information on workers employed after the discontinuation of the confounding factors. Therefore, we have updated the study at the Nitro plant with 9 more years of vital status follow up to allow us to re-examine cancer rates among MBT workers.

Materials and methods

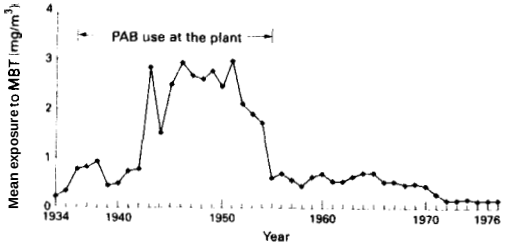
PLANT HISTORY

The Nitro plant started operations in 1922. The plant has produced or used agricultural chemicals, paper chemicals, plasticisers, fine chemicals, intermediates, and rubber chemicals, including MBT and PAB. This plant has been the subject of several epidemiology studies.[4–10] The production of MBT began at the Nitro plant in 1934 and continues to the present day. 2-Mercaptobenzothialzole is made by combining aniline, sulphur, and carbon disulphide. The production process has changed little although engineering changes have reduced exposure. The MBT from this process has been sold as a product, and converted to a sodium salt which has been used in other products made at the plant.

4-Aminobiphenyl was shipped to the Nitro plant from 1935 to 1955 and was used to make rubber antioxidants. The manufacturing process involved reacting PAB with acetone in the presence of a catalyst. High rates of bladder cancer at this plant have been attributed to exposure to PAB.[4 5 10]

STUDY GROUP

We used the same study group presented in the study of Strauss *et al*.[3] The study population was assembled from plant payroll, work



Estimated average exposure to MBT for workers exposed to MBT by year with the years of PAB manufacture highlighted.

history, and social security tax records. The study population includes the 1059 full time, white male production workers who were paid an hourly rate for ≥1 day at the Nitro plant between 1955 and 1977. Workers who left employment before 1955 were not included in this study because much of the work history information was missing. Salaried workers were excluded because of limited exposure. Thirty one women and 32 non-white men were excluded because of small numbers.

METHODS OF ASSESSING EXPOSURE

2-Mercaptobenzothialzole is not very volatile so most of the exposure would be from dusts. Dermal exposure was not considered an important route of exposure due to the nature of the material and historical use of boots, gloves, and aprons to protect the skin. We estimated exposure to MBT using the method of Strauss *et al*.[1] The MBT workers' estimated average exposure for the years 1934–77 is shown in the figure. The highest estimated exposures occurred between the period of 1943–54 when the average exposures generally exceeded 2 mg/m^3. The average estimated exposures for workers in this study were low during the first few years of production and after 1970.

The potential for exposure to PAB during the 20 years of manufacturing was limited to several areas where control analysis samples were taken, where distillation residues were removed, and where leaks occurred. Repairs to equipment and accidental leaks rather than normal operating conditions resulted in the greatest exposures.[1] Most workers had some jobs with plantwide responsibilities and therefore had the potential to be involved in the clean up of spills. As we had no way of determining which workers were involved in the clean ups of PAB, estimates of exposure to PAB were impossible. We used two definitions for exposure to PAB as workers could be exposed either in the processes which used PAB or clean ups of accidental releases. A worker was considered to have potential exposure to PAB if he either had a job in a department where exposure to PAB was possible, or was employed between 1935 and 1955, the years when PAB was used in the plant. Use of PAB at the plant coincided with the highest mean exposures of the MBT workers between 1935 and 1955 (figure).

VITAL STATUS FOLLOW UP

We obtained vital status of workers from the beginning of 1955 to the end of 1996 from company payroll and pension files, Social Security Administration, the National Death Index, Motor Vehicle Bureau of West Virginia, and credit bureau records. Further details of how the data for these workers were assembled was presented previously.[1]

At the end of the follow up period, 659 workers (62%) were verified as living, 393 (37%) were confirmed dead, and seven (1%) were lost to follow up. We obtained 99% of death certificates (388 of 393). Two nosologists coded underlying cause of death according to the rules of the eighth revision of the *international classification of diseases (ICD-8)*. The nosologists resolved discrepancies in coding through discussion. Also, the National Death Index provided underlying cause of death for six decedents on whom we had not obtained the death certificate.

ANALYTICAL METHODS

Person-time for study subjects was accumulated across 5 year age and calendar year specific intervals. Accumulation of person-time began at the hire date or 1 January 1955, whichever was the latest. Accumulation of person-time ended at the end of the study (31 December 1996), the last day worked if lost to follow up, or the date of death, whichever was the earliest. We calculated standardised mortality ratios (SMRs) to compare mortalities with the white male population of the four counties of West Virginia (Caball, Kanawha, Lincoln, and Putnam) within a 20 mile radius of the plant.[11] We used Fisher's exact method for calculation of 95% confidence intervals (95% CIs).[12] We followed guidelines for study conduct given by the Chemical Manufacturer's Association.[13]

We used cumulative exposure to MBT to evaluate the exposure-response for lung cancer, prostate cancer, and bladder cancer. Cumulative years of exposure are the sum of daily exposures divided by 356 days. Estimated exposures were cumulated before 1955 for workers who were active in 1955. The four categories of cumulative exposure were none, 0.01–1.9 mg/m^3-years, 2–7.9 mg/m^3-years, and 8–129 mg/m^3-years. Person-years for workers not exposed to MBT and those who worked before exposure to MBT began are included in the none category. These exposure categories are the same as those of the earlier study and were developed by dividing the person-years into three equal cumulative exposure categories.[1] A linear trend test was used to evaluate exposure-response.[14] Rates were also evaluated by time since first exposure (<20 years and ≥20 years).

The SMRs are presented for the total plant population and those workers exposed to MBT. The group of workers exposed to MBT is also stratified by workers with and without a potential exposure to PAB to find the modifying effect of exposure to PAB .

Table 1 Standardised mortality ratios (SMRs, (95% CIs)), observed (obs) and expected (exp) deaths for selected causes of death for original study of 1059 white hourly Nitro plant workers and update period

Cause of death (ICD-8)	Strauss et al[1] 1955–87 SMR (95% CI) [obs/exp]	Data added by update 1988–96 SMR (95% CI) [obs/exp]	Total 1955–96 SMR (95% CI) [obs/exp]
All causes (0-999)	0.9 (0.8 to 1.0) [272/313.6]	1.0 (0.9 to 1.3) [121/115.8]	0.9 (0.8 to 1.0) [393/429.4]
All cancer (140-209)	1.2 (0.9 to 1.5) [78/66.4]	0.7 (0.5 to 1.0) [26/37.3]	1.0 (0.8 to 1.2) [104/103.7]
Buccal cavity and pharyngeal (140-149)	0.6 (0.0 to 3.5) [1/1.6]	0.0 (0.0 to 5.4) [0/0.7]	0.4 (0.0 to 2.5) [1/2.25]
Oesophageal (150)	0.0 (0.0 to 2.4) [0/1.6]	0.0 (0.0 to 3.8) [0/1.0]	0.0 (0.0 to 1.5) [0/2.5]
Stomach (151)	0.5 (0.0 to 2.5) [1/2.3]	1.4 (0.0 to 7.7) [1/0.7]	0.7 (0.1 to 2.4) [2/3.0]
Colon (153)	0.6 (0.1 to 1.8) [3/4.8]	0.7 (0.1 to 2.7) [2/2.7]	0.7 (0.2 to 1.6) [5/7.5]
Rectal (154)	0.7 (0.0 to 4.0) [1/1.4]	0.0 (0.0 to 5.5) [0/0.7]	0.5 (0.0 to 2.7) [1/2.1]
Liver (155-156)	1.0 (0.0 to 5.4) [1/1.0]	0.9 (0.0 to 4.9) [1/1.1]	0.9 (0.1 to 3.3) [2/2.2]
Pancreatic (157)	0.3 (0.0 to 1.8) [1/3.0]	0.0 (0.0 to 2.5) [0/1.5]	0.2 (0.0 to 1.2) [1/4.5]
Lung (162)	1.2 (0.8 to 1.7) [32/26.3]	0.6 (0.3 to 1.2) [9/14.9]	1.0 (0.7 to 1.3) [41/41.2]
Prostate (185)	1.8 (0.7 to 3.7) [7/3.9]	0.3 (0.0 to 1.5) [1/3.7]	1.1 (0.5 to 2.1) [8/7.6]
Kidney (189.0-189.2)	0.0 (0.0 to 2.3) [0/1.6]	1.2 (0.0 to 6.6) [1/0.8]	0.4 (0.0 to 2.3) [1/2.5]
Bladder (188,189.9)	8.0 (4.2 to 13.6) [13/1.6]	3.3 (0.7 to 9.7) [3/0.9]	6.3 (3.6 to 10.3) [16/2.5]
Central nervous system (191-192)	0.6 (0.0 to 3.5) [1/1.6]	0.0 (0.0 to 4.9) [0/0.8]	0.4 (0.0 to 2.4) [1/2.3]
Leukaemia (204-207)	0.9 (0.1 to 3.1) [2/2.4]	1.3 (0.2 to 4.8) [2/1.5]	1.0 (0.3 to 2.6) [4/3.9]
All other cancers	1.1 (0.6 to 1.9) [15/13.4]	1.0 (0.4 to 2.1) [6/6.3]	1.1 (0.6 to 1.6) [21/19.7]
All heart disease (390-398,400.1,400.9,402,404,410-414,420-429)	1.0 (0.8 to 1.2) [127/129.3]	1.1 (0.8 to 1.4) [44/41.5]	1.0 (0.9 to 1.2) [171/170.8]
Ischaemic heart disease (410-414)	1.1 (0.9 to 1.3) [116/108.8]	1.9 (1.3 to 2.8) [30/15.6]	1.2 (1.0 to 1.4) [146/124.4]
Non-malignant respiratory disease (460-519)	0.6 (0.3 to 1.0) [13/22.1]	1.4 (0.8 to 2.2) [17/12.5]	0.9 (0.6 to 1.2) [30/34.6]
External causes (800-999)	0.7 (0.5 to 1.1) [22/30.5]	1.5 (0.8 to 3.8) [4/2.7]	0.8 (0.5 to 1.1) [26/33.2]
All other causes	0.5 (0.3 to 0.6) [32/65.3]	1.4 (0.9 to 2.0) [30/21.8]	0.7 (0.5 to 0.9) [62/87.1]
Person years	23943	6404	30347
Missing death certificates	1	4	5

Results

Table 1 shows the SMRs for all workers for the original study of Strauss *et al*,[1] the SMRs for the 9 year update, and the results for both groups combined. The original study had 23 943 person-years of observation versus 6404 in the update. There were 272 deaths reported in the original study versus 313.6 expected deaths (SMR=0.9, 95% CI 0.9 to 1.3). The observed numbers of deaths from lung cancer (SMR=1.2, 95% CI 0.8 to 1.7), prostate cancer (SMR=1.8, 95% CI 0.7 to 3.7), bladder cancer (SMR=8.0, 95% CI 4.2 to 13.6), and ischaemic heart disease (SMR=1.1, 95% CI 0.9 to 1.3) were greater than expected. The 1988–96 update added 121 additional deaths versus 115.8 expected deaths (SMR=1.0, 95% CI 0.9 to 1.3). The observed causes of death for the update period were at expected levels for most causes with the exception of bladder cancer (SMR=3.3, 95% CI 0.7 to 9.7), ischaemic heart disease (SMR=1.9, 95% CI 1.3 to 2.8), and non-malignant respiratory disease (SMR=1.4, 95% CI 0.8 to 2.2).

In the final column of table 1, we combine the follow up from the study of Strauss *et al* with the follow up from the update of 1988–96.[1] For the entire follow up period, the SMR for all causes of death was 0.9 (95% CI 0.8 to 1.0). Although the number of all cancers in the original study was greater than expected (SMR=1.2, 95% CI 0.9 to 1.5), the number of cancers in the combined follow up was at expected levels (SMR=1.0, 95% CI 0.8 to 1.2). For the entire follow up period, lung cancer (SMR=1.0, 95% CI 0.7 to 1.3), prostate cancer (SMR=1.1, 95% CI 0.5 to 2.1), and the other cancer sites examined were at expected levels. Among the cancers, only rates of bladder cancer were greater than expected for the entire follow up period (SMR=6.3, 95% CI 3.6 to 10.3). Also, the SMR for ischaemic heart disease for the entire follow up period was 1.2 (95% CI 1.0 to 1.4). The remaining tables present results from the combined follow up periods only.

Table 2 shows the SMRs for the workers exposed to MBT and stratified by potential exposure to PAB. The SMR for total cancer among MBT workers was similar to the entire study group and to the population living near the plant (SMR=1.0, 95% CI 0.8 to 1.3). Rates for lung cancer (SMR=1.0, 95% CI 0.7 to 1.5) and prostate cancer (SMR=0.9, 95% CI 0.2 to 2.3) were also at expected levels. The SMR for bladder cancer (SMR=8.9, 95% CI 4.7 to 15.2) was greater than expected when compared with the external reference group. The number of total cancers was greater than expected (SMR=2.0, 95% CI 1.3 to 3.0) for MBT workers with one or more job with exposure to PAB during their career. The numbers

Table 2 Standardised mortality ratios (SMRs, (95% CIs)), observed (obs) and expected (exp) deaths for workers exposed to MBT and stratified by the potential exposure to PAB

Cause of death	All MBT workers SMR (95%CI) [obs/exp]	MBT workers with jobs with exposure to PAB SMR (95%CI) [obs/exp]	MBT workers without jobs with exposure to PAB* SMR (95%CI) [obs/exp]	MBT workers with no exposure to PAB (employed after 1955) SMR (95%CI) [obs/exp]
All cancers	1.0 (0.8 to 1.3) [63/63.9]	2.0 (1.3 to 3.0) [23/11.5]	0.8 (0.5 to 1.0) [40/52.3]	0.3 (0.1 to 0.9) [3/9.7]
Lung	1.0 (0.7 to 1.5) [27/26.1]	2.4 (1.2 to 4.3) [11/4.6]	0.7 (0.4 to 1.2) [16/21.5]	0.6 (0.1 to 1.9) [2/3.6]
Prostate	0.9 (0.2 to 2.3) [4/4.4]	0.0 (0.0 to 4.4) [0/0.8]	1.1 (0.3 to 2.9) [4/3.6]	0.0 (0.0 to 11.9) [0/0.3]
Bladder	8.9 (4.7 to 15.2) [13/1.5]	27.1 (11.7 to 53.4) [8/0.3]	4.3 (1.4 to 10.0) [5/1.2]	0.0 (0.0 to 24.70 [0/0.2]
Workers	600	89	511	270
Person-years	17344	2405	14940	7405
Missing death certificates	2	1	1	0

*Includes workers who held plantwide jobs with potential exposure to PAB.

Table 3 Standardised mortality ratios (SMRs, (95% CIs)), observed (obs) and expected (exp) deaths for 511 workers without occupational exposure to PAB by cumulative exposure to MBT and test for linear trend

Cause of cancer death	Cumulative exposure to MBT (mg/m³.y) among workers without occupational exposure to PAB*				
	No exposure SMR (95% CI) [obs/exp]	0.01–1.9 SMR (95% CI) [obs/exp]	2.0–7.9 SMR (95% CI) [obs/exp]	8.0–129.0 SMR (95% CI) [obs/exp]	p Value linear trend test
Lung cancer	0.8 (0.4 to 1.5) [10/12.6]	0.8 (0.2 to 2.1) [4/4.9]	0.9 (0.3 to 2.0) [6/6.4]	0.6 (0.2 to 1.3) [6/10.1]	0.47
Prostate	1.6 (0.4 to 4.0) [4/2.6]	1.3 (0.0 to 7.2) [1/0.8]	1.2 (0.0 to 6.5) [1/0.9]	1.0 (0.1 to 3.7) [2/1.9]	0.71
Bladder	1.2 (0.0 to 6.5) [1/0.9]	0.0 (0.0 to 13.8) [0/0.3]	3.5 (0.1 to 19.5) [1/0.3]	6.5 (1.8 to 16.6) [4/0.6]	0.04
Workers	726	366	257	171	
Person-years	11921	4556	5099	5284	
Missing death certificates	1	0	0	1	

*Includes workers who held plant wide jobs with potential exposure to PAB.

of lung cancers (SMR=2.4, 95% CI 1.2 to 4.3) and bladder cancers (SMR=27.1, 95% CI 11.7 to 53.4) for this group of workers were greater than expected. The MBT workers without a job with definite exposure to PAB during their careers had an observed total cancer rate (SMR=0.8, 95% CI 0.5 to 1.0) which was lower than expected mostly as a result of fewer than expected deaths from lung cancer (SMR=0.7, 95% CI 0.4 to 1.2). Observed deaths from bladder cancer (SMR=4.3, 95% CI 1.4 to 10.0), however, were greater than expected in this group based on five deaths. Workers with no potential exposure to PAB, or those workers hired after 1955, have a lower observed than expected risk of total cancer (SMR=0.3, 95% CI 0.1 to 0.9) and there were no deaths from bladder cancer (0.2 expected).

Table 3 presents the SMRs for lung, prostate, and bladder cancer by cumulative exposure for 511 MBT workers without a job with known exposure to PAB but who may have held plant wide jobs with potential exposure to PAB. The SMRs for lung cancer and prostate cancer varied around 1.0 for all exposure groupings and there was no trend with cumulative exposure. The SMRs for bladder cancer were at expected levels in the unexposed category (SMR=1.2, 95% CI 0.0 to 6.5), no deaths occurred in the low exposure category (SMR=0.0, 95% CI 0.0 to 13.8), one death occurred in the medium category (SMR=3.5, 95% CI 0.1 to 19.5), and four deaths occurred in the high exposure category (SMR=6.5, 95% CI 1.8 to 16.6). The p value for the linear trend test indicated that the increase in SMR with cumulative exposure was unlikely to be due to chance. All five deaths from bladder cancer in workers without a job with definite exposure to PAB occurred ≥20 years after first exposure to MBT (SMR=4.8, 95% CI 1.5 to 11.1). However, there was only 0.1 death expected from bladder cancer in the category of <20 years from first exposure to MBT.

Discussion

The original study on this group of MBT workers by Strauss *et al*[3] found most cancers at expected levels. However, high rates of bladder, lung, and prostate cancer among MBT workers with potential exposure to PAB were reported. We have added 9 years of data, 6404 person-years of observation, and 121 additional deaths to the study of Strauss *et al*. During the update period, low cancer rates were observed relative to both the original study and the external comparison group. The high rates of lung and prostate cancer reported in the earlier study among MBT workers are now at expected levels with the addition of these new data. The risk of lung and prostate cancer do not seem to be related to exposure to MBT as there was no trend with cumulative exposure. Further, the recently updated Ruabon study found no increase in lung or prostate cancer among MBT workers.[15] Risks of lung and prostate cancer do not seem to be related to exposure to MBT.

We observed high rates of bladder cancer among the 600 MBT workers. Many of these MBT workers had potential exposure to PAB, a potent bladder carcinogen. The manufacturing processes for MBT and PAB overlapped for 20 years, 1935–55. The highest mean exposure to MBT occurred during the period 1935–55 when PAB was used in the plant. In total 330 MBT workers either worked in the PAB department, or worked in the plant when PAB was used. Many of the group who worked in the plant when PAB was used had jobs with plantwide responsibilities. The association of exposure to MBT and bladder cancer has to be interpreted in the context of potential confounding with PAB.

Of the 13 deaths from bladder cancer which occurred among MBT workers, eight occurred among the 89 workers who held a job in the PAB department producing an SMR of 27.1 (95%CI 11.7 to 53.4). The five remaining deaths from bladder cancer occurred among the 511 MBT workers who did not have jobs listed in the PAB department on their work history. This group of workers also had increased rates of bladder cancer (SMR=4.3, 95% CI 1.4 to 10.0). If 65 of the 511 MBT workers employed when PAB was used at the plant had exposure to PAB, an SMR of 4.3 would be observed given a relative risk of 27.1 among PAB workers. The five workers who died of bladder cancer held jobs with plantwide responsibilities for 1 month, 5 months, 8 months, 20 months, and 10 years. These five workers had job titles of maintenance worker, yard labourer, or general production worker and thus may have had exposure to PAB. However, we have no definitive information that these workers were ever exposed to PAB. Although we found an association with increasing exposure to MBT and increasing risk of bladder cancer, the number of deaths in each exposure category was small, and at least some confounding with exposure to PAB seems likely given that the highest mean exposures to MBT occurred when PAB manufacturing was taking place and each worker held a job where exposure to PAB was possible. We found no deaths from bladder cancer among MBT workers who were employed after PAB use at the plant was discontinued, although only 0.2 bladder cancers were expected in this group.

We also evaluated the potential of confounding from cigarette smoking. We used the indirect method of Axelson as data on smoking was not available from the workers at the plant.[16] We assumed that cigarette smoking would be associated with a threefold increase in the risk of bladder cancer, and that the percentage of smokers was twice as great (60% *v* 30%) in the workers exposed to MBT as the comparison population. In this situation, cigarette smoking would explain only a 38% increase in the SMR for this study population. Confounding with cigarette smoking could not be solely responsible for the observed increase in risk of bladder cancer.

In the study of the MBT workers in Ruabon, Wales by Sorahan *et al*,[15] there were seven deaths from bladder cancer (SMR=4.1, 95% CI 1.7 to 8.5). Four of the deaths occurred in the lowest category of exposure to MBT (relative risk (RR)=3.5, 95% CI 1.1 to 10.6), two in the medium exposure category (RR=2.6, 95% CI 0.6 to 11.3), and one in the highest exposure category (RR=2.5, 95% CI 0.3 to 19.4). As in our study, the authors were concerned about the confounding with exposure to phenyl-β-naphthylamine and o-toluidine which may increase the risk of bladder cancer.

In our examination of bladder cancer, we used underlying cause of death to estimate the risk. We assumed that the relative risk of death was equal to the relative risk of incidence in exposed workers. Sorahan *et al*[15] used incidence and mortality data to provide more precise estimates of risk. Collection of bladder cancer incidence data in our study, however, was not feasible.

An important limitation in the studies of both Nitro and Ruabon is the inability to evaluate risk of bladder cancer from exposure to MBT because of overlapping exposure of potent bladder carcinogens. Both PAB and β-naphthylamine have been associated with large excesses in bladder cancer after relatively short exposures.[4 5 10 17] Evaluating risk of bladder cancer from other occupational exposures in these settings is difficult if not impossible at the present time given the small number of workers without exposure to bladder carcinogens. Assessment of the potential risk of MBT for bladder cancer will have to wait for further updates of the workers at Nitro and Ruabon employed after these bladder carcinogens are no longer in the plant, or completion of studies of workers exposed to MBT without confounding exposure to bladder carcinogens.

Conclusion

The MBT workers have cancer rates that are lower than or equal to local population rates. Rates of bladder cancer are greater than expected among MBT workers, but an unknown portion of these workers had exposure to PAB. The potential confounding exposure to PAB, a potent bladder carcinogen, in an unknown portion of the MBT workers makes it impossible to evaluate risk of bladder cancer in this population. Although the lack of bladder cancers among workers hired after PAB manufacture ended would argue against a relation with exposure to MBT, the number of expected deaths in this group of workers is too small to be conclusive. However, exposure to MBT does not seem to increase the risk of most cancers including cancers of the lung and prostate.

This work was sponsored by Solutia, 10300 Olive Boulevard, St Louis, Missouri 63166, USA.

1 National Toxicology Program. *Toxicology and carcinogenesis studies of 2-mercaptobenzothiazole(CAS No 149 30 4) in F344/N rats and B6C3F1 mice. NTP/TR 332.* Research Triangle Park, NC: National Institute of Health, 1988. (No 88-2588.)

2 Sorahan T, Pope D. Mortality study of workers employed at a plant manufacturing chemicals for the rubber industry: 1955–86. *Br J Ind Med* 1993;50:998–1002.

3 Strauss ME, Barrick ED, Bannister RM. Mortality experience of employees exposed to 2-mercaptobenzothiazole at a chemical plant in Nitro, West Virginia. *Br J Ind Med* 1993;50:888–93.

4 Melick WF, Escue HM, Naryka JJ, *et al*. The first reported cases of human bladder tumors due to a new carcinogen, xenylamine. *J Urol* 1955;74:700–6.

5 Melick WF, Naryka JJ, Kelly RE. Bladder cancer due to exposure to para-aminobiphenyl: a 17-year follow up. *J Urol* 1971;106:220–6.

6 Zack JA, Suskind RR. The mortality experience of workers exposed to tetrachlorodibenzodioxin in a trichlorophenol process accident. *J Occup Med* 1980;22:11–14.

7 Zack JA, Gaffey WR. A mortality study of workers employed at the Monsanto company plant in Nitro, West Virginia. In: Tucker RE, Young AL, Grey AP, eds. *Human and environmental risks of chlorinated dioxins and related compounds*. New York: Plenum Press, 1983:565–91.

8 Suskind RR, Hertzberg VS. Human health effects of 2,4,5-T and its toxic contaminants. *JAMA* 1984; 251 2372–80.

9 Moses M, Lilis R, Crow KD, *et al*. Health status of workers with past exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin in the manufacture of 2,4,5-trichlorophenoxyacetic acid: comparison of findings with and without chloracne. *Am J Ind Med* 1984;5:161–82.

10 Collins JJ, Strauss ME, Levinskas GJ, *et al*. The mortality experience of workers exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin in a trichlorophenol process accident. *Epidemiology* 1993;4:7–13.

11 Marsh GM, Preininger M. OCMAP. A user oriented occupational cohort mortality analysis program. *American Statistician* 1980;34:245.

12 Rothman KJ, Boice JD. Epidemiologic analysis with a programmable calculator. Washington, DC: United States Government Printing Office, 1979:30–1. (DHEW Publ No (NIH)79–1649.)

13 Chemical Manufacturers Association's Epidemiology Task Group. Guidelines for good epidemiology practices for occupational and environmental epidemiologic research. *J Occup Med* 1991;33:1221–9.

14 Breslow NE, Day NE. Statistical methods in cancer research: Vol II. The design and analysis of cohort studies. Lyon: International Agency for Research on Cancer, 1987. (IARC Sci Publ No 82.)

15 Sorahan T, Hamilton L, Jackson JR. A further cohort study of workers employed at a plant manufacturing chemicals for the rubber industry, with special reference to the chemicals 2-mercaptobenzothiazole (MBT), aniline, phenyl-β-naphthylamine and ortho-toluidine. Draft Report January 11, 1999.

16 Axelson O, Aspects of confounding in occupational health epidemiology. *Scand J Work Environ Health* 1978;4:85–9.

17 Case RAM, Hosker ME, McDonald DB, *et al*. Tumours of the urinary bladder in workmen engaged in the manufacture and use of certain dyestuff intermediates in the British chemical industry, part I. The role of aniline, benzidine, α-naphthylamine, and β-naphthylamine. *Br J Ind Med* 1954;11:75–104.

Occup Environ Med 1999;56:672–673

Cancer incidence in a cohort of Swedish sewage workers: extended follow up

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Abstract

***Objectives*—To study the cancer incidence in a cohort of Swedish sewage workers. An increased incidence of cancer of the stomach, the kidney and the nervous system in this cohort was previously reported. This new analysis reports on 9 more years of follow up.**

***Methods*—The study is an analysis of a cohort of all 711 employees at 17 Swedish sewage plants employed for at least for 1 year during the years 1965–86. Assessment of exposures was performed by classification of work tasks. Standardised incidence ratios (SIRs), and 95% confidence intervals (95% CIs) were calculated.**

***Results*—The total cancer incidence was not significantly increased (SIR=1.2, 95% CI 0.92 to 1.5) but the incidence of prostate cancer was (SIR=1.6, 95% CI 1.0 to 2.5), and based on two cases only, there seemed to be a significant increase of cancer of the nose and the nasal sinuses (SIR=12, 95% CI 1.5 to 44). The incidence of stomach cancer was also increased (SIR=2.3, 95% CI 0.99 to 4.5). There was no relation between cancer incidence and level of sewage exposure.**

***Conclusions*—Sewage workers did not have an increased risk of cancer, and the increased risk estimates for some specific cancer sites were not conclusive.**

(*Occup Environ Med* 1999;56:672–673)

Keywords: cancer; epidemiology; sewage workers

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Accepted 10 June 1999

Sewage workers are exposed to a wide variety of materials including mutagenic and carcinogenic substances.[1–3] There are several reports on workers exposed to sewage that indicate an increased risk of cancer at various sites: larynx[4]; liver[4]; stomach[4–6]; urinary organs[4 6]; and lymphopoietic system.[5] In 1993 we reported the results from a retrospective analysis of a cohort of Swedish sewage workers.[10] The incidence of all cancers did not differ from that of the general population, but there were numerical increases in cancer of the stomach, the kidney, and the nervous system. This present report accounts for an additional 9 years of follow up and a roughly doubled number of tumours in the cohort.

Subjects and methods

The cohort was formed of all sewage workers who had worked for ≥1 year during the period 1965–86 at the municipal sewage plants in 17 small and medium sized cities in central and southern Sweden. Seven hundred and twelve workers (657 men and 55 women) were identified from employment records. The median age at the start of observation was 36 years (range 16–64), and the median duration of employment at the start of observation was 11 years (range 1–49). Vital status was determined on 31 December 1995. One person with a missing national identification number was excluded, one person had emigrated, 130 had died, and 580 were still alive.

For each worker the duration of employment and main work tasks were noted. Also employment before 1965 was noted and accounted for in the analyses with an induction latency period. On the basis of a qualitative exposure classification of the work tasks, four exposure classes were identified in order of increasing exposure: laboratory work, work in the sewage processing plant, sludge pipe flushing or exhaustion, and work at sewage pump stations. Subjects with employment periods with different exposure levels were allocated to their highest exposure class.

Information on tumours diagnosed from 1965 to 1994 and coded according to the *seventh revision of the international* classification of diseases (ICD-7), was obtained from the Swedish Cancer Register. Expected incidences of cancer for the period 1965–94 were calculated with national incidences specific for calendar year, site, sex, and 5 year age groups. The date of death, a second tumour diagnosis, or emigration were used as individual end points, whichever occurred first.

Cause specific standardised incidence ratios (SIRs) were calculated in separate analyses for 0, 10, and 20 years induction latency periods from the start of exposure until the start of contribution to the person-years under risk. The corresponding 95% confidence intervals (95% CIs) were calculated according to the Poisson distribution, or to the normal distribution when the expected values were >15. The term "significant" is taken to indicate that the 95% CI of the risk estimate does not include 1.

Results

There were only three cancers among the women sewage workers, one each for mouth, breast, and thyroid. The site and sex specific risk estimates for the men are shown in the table. The total number of cancers did not differ from expected (SIR 1.2, 95% CI 0.92 to 1.5) but the incidence of prostate cancer was significantly higher than expected; 21 versus 13 expected (SIR 1.6, 95% CI 1.0 to 2.5). Based on two cases only, there seemed to be a significant increase in cancer of the nose and the nasal sinuses (SIR 12, 95% CI 1.5 to 44). The increased incidence of stomach cancer almost reached conventional levels of significance (SIR 2.3, 95% CI 0.99 to 4.5) but the incidence of cancer of the kidney and the nervous system, the two other sites discussed in our previous report, were not significantly increased.

Site specific cancer incidence (ICD-7) of male Swedish sewage workers 1965–94

	No induction-latency 13 452 person-years				*10 years induction-latency 7 756 person-years*				*20 years induction-latency 2 907 person-years*			
	O	*E*	*SIR*	*95% CI*	*O*	*E*	*SIR*	*95% CI*	*O*	*E*	*SIR*	*95% CI*
All sites (140–209)	77	66	1.2	0.92 to 1.5	65	55	1.2	0.91 to 1.5	43	32	1.3	0.98 to 1.8
Lip (140)	1	0.59	1.7	0.04 to 9.4	1	0.47	2.1	0.05 to 12	1	0.26	3.9	0.10 to 22
Mouth (141, 143, 144)	1	0.66	1.5	0.04 to 8.4	1	0.53	1.9	0.05 to 10		0.28	0	0 to 13
Salivary glands (142)	1	0.19	5.3	0.13 to 29	1	0.14	7.0	0.18 to 39	1	0.07	14	0.34 to 76
Pharynx (145–148)	1	0.56	1.8	0.05 to 10	1	0.45	2.2	0.06 to 12	1	0.23	4.4	0.11 to 24
Oesophagus (150)	2	0.86	2.3	0.28 to 8.4	1	0.74	1.4	0.03 to 7.6	1	0.42	2.4	0.06 to 13
Stomach (151)	8	3.5	2.3	0.99 to 4.5	6	2.9	2.1	0.77 to 4.6	2	1.7	1.2	0.15 to 4.3
Colon (153)	2	4.6	0.43	0.05 to 1.6	2	3.9	0.51	0.06 to 1.8	2	2.4	0.85	0.10 to 3.1
Rectum and anus (154)	4	3.2	1.3	0.34 to 3.2	4	2.7	1.5	0.41 to 3.8	3	1.6	1.9	0.40 to 5.6
Pancreas (157)	4	2.1	1.9	0.51 to 4.8	3	1.8	1.7	0.35 to 5.0	2	0.99	2.0	0.25 to 7.3
Nose and sinuses (160)	2	0.17	12	1.5 to 44	1	0.13	7.7	0.19 to 43	1	0.07	14	0.35 to 78
Trachea, bronchi, and lungs (162.0–1)	7	7.1	0.99	0.40 to 2.0	6	5.9	1.0	0.37 to 2.2	4	3.3	1.2	0.33 to 3.1
Prostate (177)	21	13	1.6	1.0 to 2.5	18	12	1.5	0.89 to 2.4	15	8.0	1.9	1.0 to 3.1
Renal parenchyma (180.0)	3	2.4	1.2	0.26 to 3.7	2	1.9	1.1	0.13 to 3.8	1	1.0	1.0	0.03 to 5.6
Urinary organs (excluding kidney, 181)	3	4.8	0.63	0.13 to 1.8	3	4.1	0.74	0.15 to 2.2	1	2.4	0.42	0.01 to 2.3
Malignant melanoma (190)	3	2.4	1.3	0.26 to 3.7	2	1.8	1.1	0.13 to 4.0		0.88	0	0 to 4.2
Skin (excluding melanoma, 191)	2	2.5	0.79	0.10 to 2.8	2	2.3	0.88	0.11 to 3.2	1	1.5	0.66	0.02 to 3.7
Nervous system (193)	4	2.3	1.8	0.48 to 4.5	3	1.6	1.8	0.37 to 5.3	2	0.78	2.6	0.31 to 9.3
Endocrine glands (195)	1	0.92	1.1	0.03 to 6.1	1	0.68	1.5	0.04 to 8.2		0.32	0	0 to 12
Multiple myeloma (203)	2	1.0	2.0	0.24 to 7.1	2	0.85	2.4	0.28 to 8.5	2	0.49	4.1	0.49 to 15
Polycythemia vera (208)	1	0.23	4.4	0.11 to 25	1	0.18	5.7	0.14 to 32	1	0.09	11	0.28 to 61

O=observed number of cancers; E=expected number of cancers.

There was no significant increase in the incidence of all cancers combined when accounting for 0, 10, and 20 years induction latency periods, respectively, counted from the beginning of the first exposure period. The risk of prostate cancer increased slightly when the 20 years induction latency was accounted for (SIR 1.9, 95% CI 1.0 to 3.1). The risk estimates for all cancers were not related to exposure in any consistent fashion and the numbers of site specific diagnoses were generally too small for meaningful analyses of exposure-response relations.

Discussion

Our finding of no increased risk for total cancer among the sewage workers was consistent with other studies.[4 6] Compared with our previous report, the increased incidence of some specific cancers was reduced whereas the increase of prostate cancer was a new observation.

Prevailing geographical differences in cancer incidence and the use of national rates as the reference may cause misinterpretation of the results of this study. Our cohort was collected from small municipalities in Sweden not including the three largest cities. These three cities differ from the rest of the country in some specific morbidities. If a disease being studied is systematically more incident in the largest cities, the real risk for the cohort could be underestimated. The incidence of lung cancer is substantially higher in the largest cities of Sweden whereas the differences for the other cancers of interest in this study are less pronounced.

The aetiology of prostate cancer is unknown and increased risk of prostate cancer has not been reported previously in sewage workers. Occupational exposure to cadmium has been discussed as a potential prostatic carcinogen, but the results from different studies have been inconsistent.[11] Municipal sewage sludge may contain cadmium, but the exposure of sewage workers is certain to be much lower than the exposures at which an increased incidence of prostate cancer has been reported.

Among the specific cancers discussed in our previous report, stomach cancer might still warrant some interest because of the remaining numerical increase of incidence and similar results reported by other research groups.[4 6] However, these reports have been inconclusive and there is no report about exposure to any specific gastric carcinogen among sewage workers.

Despite some reports of carcinogenic and mutagenic exposures among sewage workers,[1–3] there is no convincing evidence of significant carcinogenic exposures in sewage work in general. We found no consistent trend between cancer risk and exposure level, and the highest numerical risk was in fact among those with the lowest exposure. The observations of increased cancer incidences in this study are most likely to be due to chance, at least they do not indicate any large risk. Our final conclusion was that the Swedish sewage workers studied were not at increased risk for cancer due to their work.

1 Rappaport SM, Richard MG, Hollstein MC, *et al.* Mutagenic activity in organic wastewater concentrates. *Environmental Science and Technology* 1979;13:957–61.
2 Scarlett-Kranz JM, Babish JG, Strickland D, *et al.* Urinary mutagens in municipal sewage workers and water treatment workers. *Am J Epidemiol* 1986;124:884–93.
3 Babish JG, Johnson BE, Lisk DJ. Mutagenicity of municipal sewage sludges of American cities. *Environmental Science and Technology* 1983;17:272–7.
4 Lafleur J, Vena JE. Retrospective cohort mortality study of cancer among sewage plant workers. *Am J Ind Med* 1991;19:75–86.
5 Beternps EJ, Buncher CR, Clark CS. Proportional mortality analysis of wastewater treatment system workers by birthplace with comments on amyotrophic lateral sclerosis. *J Occup Med* 1994;36:31–5.
6 Rapiti E, Sperati A, Fano V, *et al.* Mortality among municipal workers in garbage handling, waste incinerating and sewage treatment: a retrospective cohort study. *Epidemiology* 1995;6(suppl 4):S135.
7 Chow W-H, McLaughlin JK, Malker HSR, *et al.* Occupation and stomach cancer in a cohort of Swedish men. *Am J Ind Med* 1994;26:11–20.
8 Cocco P, Ward MH, Dosemeci M. Occupational risk factors for cancer of the gastric cardia. *J Occup Environ Med* 1998;40:855–61.
9 Sorahan T, Hamilton L, Wallace DMA, *et al.* Occupational urothelial tumours: a regional case-control study. *Br J Urol* 1998;82:25–32.
10 Friis L, Edling C, Hagmar L. Mortality and incidence of cancer among sewage workers: a retrospective cohort study. *Br J Ind Med* 1993;50:653–7.
11 Järup L, Nordberg G. Cancer. In: Järup L, Berglund M, Elinder CG, *et al*, eds. Health effects of cadmium exposure: a review of the literature and a risk estimate. *Scand J Work Environ Health* 1998;24(suppl 1):34–6.

Occup Environ Med 1999;56:674–678

Correlation between urinary 2-methoxy acetic acid and exposure of 2-methoxy ethanol

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Abstract

***Objectives*—To examine the correlation between airborne 2-methoxy ethanol (ME) exposures and the urinary 2-methoxy acetic acid (MAA) and to recommend a biological exposure index (BEI) for ME.**

***Methods*—8 Hour time weighted average (TWA) personal breathing zone samples and urine samples before and after the shift were collected from Monday to Saturday for 27 workers exposed to ME and on Friday for 30 control workers.**

***Results*—No correlation was found between airborne exposure to ME and urinary MAA for nine special operation workers due to the use of personal protective equipment. For 18 regular operation workers, a significant correlation (r=0.702, p=0.001) was found between urinary MAA (mg/g creatinine) on Friday at the end of the shift and the weekly mean exposures of ME in a 5 day working week. The proposed BEI, which corresponds to exposure for 5 days and 8 hours a day to 5 ppm, extrapolated from the regression equation is 40 mg MAA/g creatinine. A significant correlation was also found between the weekly increase of urinary MAA (Friday after the shift minus Monday before the shift) and the weekly mean exposures of ME (r=0.741). The recommended value of the weekly increase of urinary MAA for 5 days repeated exposures of 5 ppm ME is 20 mg/g creatinine. No urinary MAA was detected in workers in the non-exposed control group.**

***Conclusions*—The Friday urinary MAA after the shift or the weekly increase of urinary MAA is a specific and a good biomarker of weekly exposure to ME.**

(*Occup Environ Med* 1999;56:674 678)

Keywords: 2-methoxy acetic acid; 2-methoxy ethanol; biological monitoring

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Accepted 14 June 1999

The solvent 2-methoxy ethanol (ME) is a widely used solvent in industries—such as the production of 2-methoxy acetate, plasticiser of 35 mm film, insulation for high voltage wires, high flash coatings, component of the photoresistant material used in photolithography of semiconductor circuit designs—in addition to being used in coating or lamination resins of circuit boards. Substantial quantities are used as de-icing additives in military jet fuel.[1 2] The annual consumption of ME is over 3000 tonnes in Taiwan. With the rapid growth of the semiconductor manufacturing industry in Asia, the quantitative exposure assessment of ME becomes increasingly important.

Animal and human studies have shown that ME can cause adverse reproductive, developmental, and haematological effects through inhalation, dermal absorption, and ingestion.[2 7] Metabolic studies showed that ME is metabolised to 2-methoxy acetic acid (MAA) through alcohol dehydrogenase and aldehyde dehydrogenase.[8 10] In humans, the total amount of MAA excreted in the urine accounts for about 85.5% of the inhaled ME.[10] In vitro studies showed that ME itself evokes no toxic responses either in Sertoli cell culture[9] or in rat whole embryo culture, whereas MAA does.[11] In vivo studies showed that equivalent doses of ME and its corresponding major metabolite, MAA, are equally effective in causing adverse effects.[9 12 13] MAA, therefore, was suggested as the proximate toxicant of ME exposure.[8 11 14 15]

The United States National Institute for Occupational Safety and Health (NIOSH) suggests that urinary MAA is a specific and good biomarker of exposure to ME.[1] Until now, however, urinary MAA has not been detected in humans except for two human subject studies in the laboratory.[10 16] The American Conference for Governmental Industrial Hygienists (ACGIH) biological exposure indices (BEIs) committee, has put ME in the adopted list of determinants of biological exposure and has tried for several years to recommend a BEI value for ME based on the committee's review of publications; however, a specific BEI could not be decided due to insufficient human data.[17]

The main purposes of this paper are to examine the correlation between the airborne exposures to ME and the internal doses (urinary MAA) and to recommend a BEI for ME.

Materials and methods

MANUFACTURING PROCESS

Two types of operations are basically performed in this study in a manufacturing plant of *semiconductor copper laminate circuit* boards. The regular operations include unwinding, splicing, accumulation, dipping, oven heating, edge trimming, rewinding, and sheeting operations. Workers were exposed to ME mainly in dipping and oven heating operations. The special operations include raw material mixing, charging, machine cleaning, and maintenance work.

SUBJECTS

A total of 27 workers, 18 in regular operations and nine in special operations, participated in this study. In special operations, two workers handled the raw material mixing, charging, and discharging, and seven workers were in charge of the cleaning and maintenance of the coating machine. Twenty workers in the administrative department and 10 workers in the heat press operations of the same plant were chosen as the control groups. All workers were in fixed work shifts and were asked not to have alcohol, medication, and other exposures of organic solvents (such as painting at home) during this study. Informed consent was given by all workers who participated in this study.

CHEMICALS USED IN THE MANUFACTURING PROCESS

The main raw materials used in this plant include epoxy and phenolic resins, hardener (dicyanamide), catalyst (2-methylimidazole), antimony oxide (Sb_2O_3), aluminum oxide, silica dioxide, ME, acetone, titanium oxide, and pigments. Acetone and ME are the only two volatile chemicals used in the workplace. The coating glue contains 70% of ME, and 30% of acetone. The qualitative confirmation of ME in raw materials were reconfirmed by gas chromatography-mass spectrometry (GC-MS G1,800A, GCD, Hewlett Packard, Palo Alto, CA, USA).

ENGINEERING CONTROL AND PERSONAL PROTECTIVE EQUIPMENT

All coating machinery was installed in enclosed rooms with aluminum sliding doors. Incinerators were installed above each coating machine to burn off the waste solvent exhaust vapours. Occasionally, workers were exposed to high concentrations of ME when the doors of the coating rooms were not closed. For the rest of the time, workers stayed in air conditioned control rooms. For the duration of this study, workers in regular operations agreed to wear protective rubber gloves to avoid contact with the glue that contained ME. All exposed workers wore short sleeved work clothes. Exposure to ME in regular operations mainly came through inhalation and skin absorption of ME vapour.

Workers in the special operations were exposed to high concentrations of ME when they went into the enclosed rooms for machine cleaning, trouble shooting, maintenance, and machine fixing. Raw materials were mixed, charged, and discharged in a separated workplace without ventilation. Workers were exposed to high concentrations of ME when the raw materials were charged, mixed in the reactors, and when manholes of the reactors were opened to charge the solid raw materials. Most workers in the special operations wore respirators and rubber gloves, but a few of them removed the respirators or gloves for a very short time during exposures. Therefore, the exposures in special operations might have come through inhalation, the inadvertent contact of liquid ME with the hands, and whole body skin absorption of ME vapour. The exposure time was short in the coating department and raw material handling area. The relative humidity in the workplaces was around 80%–85%. The temperature in the control rooms and the enclosed rooms for dipping or oven heating areas were 25–27°C and 30–33°C, respectively.

EXPOSURE MONITORING

Eight hour time weighted average (TWA) personal breathing zone samples were collected for 27 exposed workers from Monday to Saturday and for 30 workers in the control group on Friday. The Taiwan Institute of Occupational Safety and Health (IOSH) standard procedure 1215 was used to sample, measure, and analyse the airborne ME concentrations in this study.[18] This standard was fully validated with a dynamic standard gas generation system and also by three accredited industrial hygiene laboratories in Taiwan.

Personal breathing zone samples were collected by drawing air through standard size coconut shell charcoal tubes (SKC 226-01, batch No 120, PA, USA), with Gilian constant low flow personal pumps (Model LFS-114, NJ, USA), then desorbed in 1 ml of mixed solvent of 95:5 (v/v) dichloromethane (99.7%, E Merck, Darmstadt, Germany) and methanol (99.7%, E Merck). After 40 minutes shaking, the desorbed ME was analysed by a gas chromatography (GC) (Hewlett-Packard 5890 Series II, CA, USA) equipped with a HP 7673A autosampler and a flame ionisation detector.

The overall accuracy (bias plus twice the precision) was ±9.5%, the reliable measurement limit was 4 ng. The sampling flow rate was calibrated before and after each sample. All air samples were analysed by an accredited industrial hygiene laboratory in Taiwan within 1 week of sampling (samples were stored at -20°C).

BIOLOGICAL MONITORING

Urine samples before and after the shift were collected from 27 exposed workers for 6 days, and urine samples were collected after the shift from 30 workers in the control group on Friday. Urinary MAA was measured by a modified method previously reported by Sakai *et al*.[19] This method was fully validated by IOSH and also by two reference laboratories before it was adopted in this study.[20]

An internal standard of 0.1 ml butoxy acetic acid (BAA, 99.0%, E Merck, Darmstadt, Germany) was added to 1 ml urine, which was acidified with 0.1 ml concentrated hydrogen chloride. The MAA was then extracted with 1 ml of the mixture of 2:1 (v:v) dichloro-methane (99.7%, E Merck) and isopropyl alcohol (99.7%, E Merck) for 10 minutes. After centrifuging, 0.5 ml of the lower layer was transferred to a 1.5 ml vial, and 25 µl of a solution of trimethylsilyldiazomethane (TMSD, 2.0 M solution in hexane, Aldrich, WI, USA) was added. The esterified acid was analysed by a gas chromatogram equipped with an HP 7673A autosampler and a flame ionisation detector.

The MAA concentration range of the standard curve was 3.5–200 µg/ml. The limit of measurement (mean plus 10 SDs of the blank) was 0.136 µg/ml. Urinary creatinine was analysed by the Jaffe method for concentration correction. The results of urine samples were expressed as mg MAA/g creatinine. All urine samples were analysed by one reference

The 6 day time weighted average (TWA) exposures of 2-methoxy ethanol (ME) for 18 regular operation workers and nine special operation workers

Regular operation workers	Weekly exposures of ME mean (SD) ppm	Special operation workers	Weekly exposures of ME mean (SD) ppm
1	3.91 (2.66)	1	32.90 (61.97)
2	4.46 (2.38)	2	23.61 (53.16)
3	1.62 (1.50)	3	25.22 (60.13)
4	0.97 (0.58)	4	35.53 (65.97)
5	0.72 (0.73)	5	38.40 (62.33)
6	7.46 (5.62)	6	37.26 (63.97)
7	5.45 (5.44)	7	48.27 (70.43)
8	9.73 (4.36)	8	267.6 (351.4)
9	3.39 (1.83)	9	232.9 (361.8)
10	4.32 (2.44)		
11	8.86 (4.98)		
12	4.57 (5.92)		
13	1.01 (0.54)		
14	6.18 (10.2)		
15	3.97 (2.82)		
16	3.91 (2.19)		
17	6.01 (2.92)		
18	3.78 (3.12)		

The daily mean (SD) exposures of ME for 18 regular operation workers from Monday to Saturday were 3.26 (4.65), 5.76 (4.50), 4.64 (4.31), 5.31 (4.10), 5.03 (3.70), and 2.61 (2.65) ppm, respectively, with a weekly mean (SD) exposure level of 4.46 (2.56) ppm.

The daily mean (SD) exposures of ME for 9 special operation workers from Monday to Saturday were 279.2 (258.0), 152.8 (303.9), 25.60 (26.35), 10.26 (19.06), 17.38 (27.29), and 4.43 (4.76) ppm, respectively, with a weekly mean (SD) exposure level of 81.61 (111.7) ppm.

laboratory in Taiwan within 2 weeks of sampling (samples were stored at -80°C). Urinary MAA is stable for at least 5 months, and for three freeze-thaw cycles from -20°C.[20] Pooled urine samples from 12 unexposed people were used as a matrix blank and were checked for background interference.

Results

ENVIRONMENTAL MONITORING

The GC and gas chromatography/mass spectrometry (GC/MS) analysis of bulk samples and randomly selected air samples confirmed that ME and acetone were the only two volatile chemicals in the workplace.

A total of 149 8 hour TWA personal breathing zone samples were collected from 27 exposed workers. The daily mean (SD) exposures of ME for 18 regular operation workers from Monday to Saturday were 3.26 (4.65), 5.76 (4.50), 4.64 (4.31), 5.31 (4.10), 5.03 (3.70), and 2.61 (2.65) ppm, respectively. The weekly mean (SD) exposure was 4.46 (2.56) ppm (table).

For the nine special operation workers, however, the exposures were much higher. The daily mean (SD) exposures of ME from Monday to Saturday were 279.2 (258.0), 152.8 (303.9), 25.60 (26.35), 10.26 (19.06), 17.38 (27.29), and 4.43 (4.76) ppm, respectively. The weekly mean (SD) exposure was 81.61 (111.7) ppm (table). It was also noticed that peak exposures happened mainly in the first and the second day of the week. The mean (SD) peak exposures during the mixing of raw materials (on Monday and Tuesday) and in cleaning the coating machine (on Monday) were 690.7 (187.6) and 154.5 (15.4) ppm, respectively. When these peak exposures were excluded, the mean (SD) exposures of the workers who did these two operations dropped dramatically to 29.96 (28.60) and 8.87 (15.81) ppm, respectively.

No background exposures were detected in the control group workers, except for three heat press workers whose working position was closely adjacent to the coating department. The exposures of those workers were very low (the 8 hour TWA ME exposures were in the range of 0.2–0.3 ppm).

The recoveries of 20 sets of blind field samples (spiked quality control samples mixed with field samples) were in the range of 94% to 108%. The mean (SD) recoveries of proficiency analytical testing samples from three different accredited laboratories were 97.19 (3.36)%, 98.52 (2.10)%, and 97.72 (2.39)%, respectively. These results validate the reliability of the exposure monitoring data obtained in this study.

BIOLOGICAL MONITORING

Urinary MAA was confirmed by GC-MS. A total of 309 spot urine samples were collected from 27 exposed workers. Five urine samples were not included in the data analyses either due to the extreme concentrations of urinary creatinine (<0.3 g/l or >3 g/l) or low urinary MAA concentrations (<3.5 μg/ml, the lowest concentration of the standard curve).

The daily mean (SD) urinary MAA of 18 regular operation workers showed a clear increase from 10.57 (5.85) mg/g creatinine on Monday before the shift to 46.46 (33.49) mg/g creatinine on Friday after the shift (figure 1 A) even though their daily mean airborne exposures were consistent and close to the current 5 ppm permissible exposure limit (PEL).

It was surprising to notice that the daily mean urinary MAA of the highly exposed workers in special operations was even lower than that in regular operations. The daily mean (SD) urinary MAA of workers in special operations also increased clearly from 10.17 (9.00) mg/g creatinine on Monday before the shift to 29.55 (17.72) mg/g creatinine on Friday after the shift (fig 1 B).

No background urinary MAA was detected in control workers except for the three heat press workers already mentioned. The urinary

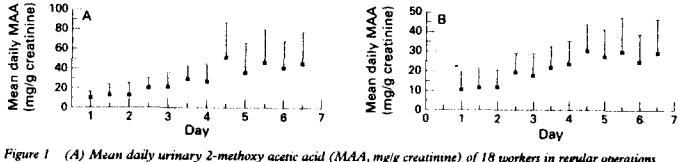

Figure 1 (A) Mean daily urinary 2-methoxy acetic acid (MAA, mg/g creatinine) of 18 workers in regular operations from Monday to Saturday. (B) Mean daily urinary MAA of 9 workers in special operations from Monday to Saturday. Data are means (SDs).

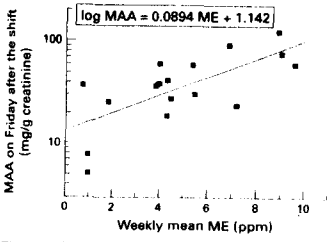

Figure 2 Correlation between the urinary MAA on Friday after the shift and the mean weekly TWA exposures to ME.

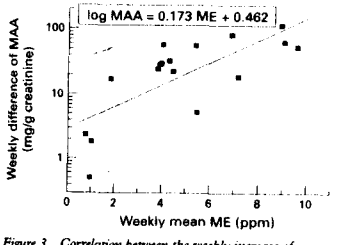

Figure 3 Correlation between the weekly increase of urinary MAA (Friday after the shift to Monday before the shift) and the mean weekly TWA exposures to ME.

MAA concentrations (0.4–0.5 μg/ml) of these workers were well below the lowest concentration of the calibration curve (3.5 μg/ml).

A total of 64 sets of triplicate analysis of urinary concentrations of MAA showed a pooled coefficient of variation of 3.07% (0.30%–7.76%) over the concentration range of 6.27–238.77 μg/ml. No interfering chemicals were found in GC and GC-MS results of randomly selected urine samples. The recoveries of 20 sets of blind quality control samples were from 93.23% to 113.75%. These results validate the reliability of the biological data.

CORRELATION BETWEEN THE AIRBORNE EXPOSURES TO ME AND THE INTERNAL DOSES

No correlation was found ($p>0.3$) between the weekly mean exposures of ME and the urinary MAA on Friday after the shift for all the 27 exposed workers.

When the workers in special operations were excluded, a significant correlation was then found between the urinary MAA on Friday after the shift and the weekly mean exposures to ME (log MAA (mg/g creatinine) $=1.142+0.089\times$ME (ppm), $p=0.001$, $r=0.702$ (fig 2)). The correlation coefficients were reduced from 0.702 to 0.601 when urinary MAA concentrations were not corrected by creatinine.

A similar correlation was found between the weekly mean exposures to ME and the weekly increase of urinary MAA (Friday after the shift minus Monday before the shift) (log MAA (mg/g creatinine)$=0.173\times$ME (ppm)$+0.462$, $p=0.0007$, $r=0.741$ for a 5 day working week (fig 3)).

Discussion

To the best of our knowledge, this is so far the only study that examines the correlation between airborne exposures to ME and the internal dose (urinary MAA) and the recommended BEI of ME in the literature.

The NIOSH has recommended that if end of shift urinary MAA exceeds 0.8 mg/g creatinine, then there is evidence of occupational exposure to ME.[1] This value was extrapolated from Groeseneken's 4 hour human experimental exposure by inhalation[10] for 4–50 minute periods at rest to 5.1 ppm, to an 8 hour exposure by inhalation to 0.1 ppm at 60 watts of exercise. Although 5 ppm is currently the most popular PEL for ME in the world, NIOSH has not recommended a BEI corresponding to this concentration.

Several points need to be discussed for the above recommendation. Firstly, the recommended value was based on exposures through inhalation only. A recent human subject study showed that the dermal absorption of vaporous ME could cause an even higher uptake than exposure to the same dose through inhalation.[16] The absorption of ME vapour through the skin was unavoidable in the workplaces. Secondly, the half life of urinary MAA in humans was 77.1 hours.[10] Our data in this field study have shown a clear accumulation of urinary MAA in workers repeated exposed daily. Values based only on single exposure through inhalation would result in an underestimated internal dose. In this study, correlations between the weekly mean exposures of ME and the urinary MAA on Friday after the shift and the weekly increase of urinary MAA were examined. The recommended BEI proposed in this way not only considered both the inhalation exposures and the absorption of vapour through the skin, but also accounted for the accumulation of internal dose in repeated daily exposures.

In this study, a significant correlation between the weekly mean exposures of ME and the Friday urinary MAA after the shift was found in workers in regular operations. This was consistent with the suggestions of the United States ACGIH.[17] The recommended BEI of urinary MAA corresponding to exposures from 5 consecutive days full shifts to a concentration of ME in air of 5 ppm extrapolated from the linear regression was 40 mg (0.45 mmol) MAA/g creatinine. The correction of creatinine provided a better correlation. Veulemans *et al* in their field study of 2-ethoxy ethanol (EE) have proposed a BEI of 150 mg (1.47 mmol) 2-ethoxy acetic acid (EAA)/g creatinine for exposures of EE at 5 ppm.[21] The ACGIH recommended BEI for EE is 100 mg (0.98 mmol) EAA/g creatinine.[17] A more stringent recommended BEI for ME obtained from this study is also reasonable due to the higher toxicity of ME. In this study, the daily exposures during regular operations were stable and close to the current PEL (5 ppm) during the working week. Workers basically only performed light physical work with short sleeved working clothes and no direct contact of liquid ME. The exposures came mainly from inhalation and

absorption of vapour through the skin. Both air and biological samples were randomly reconfirmed by GC/MS, which is one of the most specific and powerful methods for analyses of organic compounds. The good quality control programme and high recoveries of quality control and blind samples confirmed that both the environmental monitoring and biological monitoring data in this study were valid.

As the half life of urinary MAA in humans is 77.1 hours,[10] high previous exposures may result in a considerable increase in the baseline urinary MAA during the next week. It is therefore recommended that the weekly increase of urinary MAA between the end of the shift at the end of the working week and before the shift on Monday may be an alternative biomarker to reflect the weekly exposures of ME. The recommended value of this weekly increase of urinary MAA (corresponding to 5 continuous days of exposure to 5 ppm ME) extrapolated from our linear regression (fig 3) is 20 mg/g creatinine.

For special operations, the correlation between external exposures and internal doses was much more complicated and difficult to establish than that in regular operations. For most of the workers in special operations, the external exposures were extremely high, but the actual uptake was low mainly due to the proper use of butyl rubber gloves and charcoal cartridge respirators. Therefore, the workers in special operations were well protected with effective personal protective equipment. The Council of Labor Affairs also had the authority to make the employer and employees cooperate and follow the good working practises and safety guidelines and stay for only a short time in areas of high exposure during this study. On the other hand, the heavy work carried out in loading could cause more sweat to wet the body surface and working clothes (ME is highly water soluble and permeable to the skin). Short periods of inadvertent skin contact with glue (containing ME) was noted. Whole body skin absorption of ME *vapour was also difficult to avoid. All these* factors made the prediction of internal dose in workers in special operations very difficult. In situations like this, the traditional environmental monitoring might provide false information on the actual exposure to ME. The internal dose, on the other hand, can provide better information on the actual uptake and reflect the concentrations in the target sites.

It was noticed that the non-peak exposures in workers doing special operations were much lower, but the urinary MAA was significantly higher than that in peak exposures. This probably could be explained by the proper use of personal protective equipment in peak exposures (Monday) and the considerable accumulation of internal dose during the non-peak exposures (Tuesday to Saturday).

No background urinary MAA was detected in non-exposed workers in the control group, consistent with other reports.[1,2] Only low urinary MAA (0.4–0.5 μg/ml) was detected for three workers with low background exposures to ME (0.2–0.3 ppm). This further validated the specificity of urinary MAA as the biomarker of exposure to ME.

It is therefore concluded that urinary MAA is a specific and a good biomarker of the exposures to ME. The recommended BEI obtained *in this study was 40 mg MAA/g* for urinary MAA on Friday after the shift that corresponds to exposures from 5 consecutive days full shifts to a concentration of ME air of 5 ppm creatinine. The recommended value of the weekly increase of urinary MAA corresponding to 5 consecutive days full shifts to a concentration of ME air of 5 ppm creatinine was 20 mg/g creatinine.

We are grateful to Dr On-Chih Hsieh and Dr Guo-Dong Liow for their assistance in the collection of urine samples, and Dr Jing-Shiang Hwang for the statistical analyses of data. This work was supported by the Institute of Occupational Safety and Health, Council of Labor Affairs, Executive Yuan, Taiwan, Republic of China (IOSH grant 85-A304).

1 National Institute of Occupational Safety and Health. *Criteria for a recommended standard: occupational exposure to ethylene glycol monomethyl ether, ethylene glycol monoethyl ether, and their acetates.* Washington, DC: NIOSH, 1991. (Publ No 91-119).
2 Occupational Safety and Health Administration. *Occupational exposure to 2-methoxy ethanol, 2-ethoxy ethanol, and their acetates (glycol ethers); proposed rule. Federal Register.* Washington, DC: OSHA, 1993. (29CFR, Vol 58, No 54, Docket No H-044.)
3 Nagano K, Nakayama E, Oobayashi H, *et al.* Experimental studies on toxicity of ethylene glycol monomethyl ether in Japan. *Environ Health Perspect* 1984;57:75–84.
4 Larese F, Fiorito A, Zotti RD. The possible haematological effects of glycol monomethyl ether in a frame factory. *Br J Ind Med* 1992;49:131–3.
5 Wickramaratne GA. The teratogenic potential and dose-response of dermally administered ethylene glycol monomethyl ether estimated in rats with the Chernoff-Kavlock assay. *J Appl Toxicol* 1986;6:165–6.
6 Welch LS, Schrader SM, Turner TW, *et al.* Effects of exposure to ethylene glycol ethers on shipyard painters: II. Male reproduction. *Am J Ind Med* 1988;14:509–26.
7 Welch LS, Cullen MR. Effects of exposure to ethylene glycol ethers on shipyard painters: III. Hematological effects. *Am J Ind Med* 1988;14:527–36.
8 Miller RR, Hermann EA, Langvardt PW, *et al.* Comparative metabolism and disposition of ethylene glycol monomethyl ether and propylene glycol monomethyl ether in male rats. *Toxicol Appl Pharmacol* 1983;67:229–37.
9 Moss EJ, Thomas LV, Cook AW, *et al.* The role of metabolism in 2-methoxy ethanol induced testicular toxicity. *Toxicol Appl Pharmacol* 1985;79:480–9.
10 Groesenecken D, Veulemas H, Masschelein R, *et al.* Experimental human exposure to ethylene glycol monomethyl ether. *Int Arch Occup Environ Health* 1989;61:243–7.
11 Yonemoto J, Brown NA, Webb M. Effects of dimethoxyl phthalate, monomethoxyethyl phthalate, 2-methoxyethanol and methoxyacetic acid on post-implantation rat embryos in culture. *Toxicol Lett* 1984;21:97–102.
12 Miller RR, Carreon RE, Young JT, *et al.* Toxicity of methoxyacetic acid in rats. *Fundam Appl Toxicol* 1982;2:158–60.
13 Sleet RB, Greene JA, Welsch F. The relationship of embryotoxicity to disposition of 2-methoxyethanol in mice. *Toxicol Appl Pharmacol* 1988;93:195–207.
14 Brown NA, Holt D, Webb M. The teratogenicity of methoxyacetic acid in the rat. *Toxicol Lett* 1984;22:93–100.
15 Rutter EJ, Scott WJ Jr, Randall JL, *et al.* Teratogenicity of dimethoxyethyl phthalate and its metabolites methoxy ethanol and methoxyacetic acid in the rat. *Teratology* 1985;32:25–31.
16 Kezic S, Mahieu K, Monster A, *et al.* Dermal absorption of vaporous and liquid 2-methoxyethanol and 2-ethoxyethanol in volunteers. *Occup Environ Med* 1997;54:38–43.
17 American Conference of Governmental Industrial Hygienists. *TLVs threshold limit values for chemical substances and physical agents and biological indices (BEIs).* Cincinnati, OH: ACGIH; 1998:97–101.
18 Institute of Occupational Safety and Health. *Recommended sampling and analytical methods for 2-methoxy ethanol (method 1215).* Taipei, Taiwan, Republic of China: IOSH, 1992.
19 Skai T, Araki T, Matuyama Y. Determination of urinary alkoxy acetic acids by a rapid and simple method for biological monitoring of workers exposed to glycol ethers and their acetates. *Int Arch Occup Environ Health* 1993;64:495–8.
20 *Chang MJ, Shih TS, Chou JS.* Recommended biological monitoring method to measure urinary alkoxy acetic acids. Taipei, Taiwan, Republic of Taiwan: IOSH, 1992.
21 Veulemans H, Groesenecken D, Masschelein R, *et al.* Field study of the urinary excretion of ethoxy acetic acid during repeated daily exposure to the ethyl ether of ethylene glycol and ethyl ether of ethylene glycol acetate. *Scand J Work Environ Health* 1987;13:239–42.

Air pollution and hospital admissions for respiratory and cardiovascular diseases in Hong Kong

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Accepted 13 May 1999

Abstract

***Objective*—To investigate short term effects of concentrations of pollutants in ambient air on hospital admissions for cardiovascular and respiratory diseases in Hong Kong.**

***Methods*—Retrospective ecological study. A Poisson regression was performed of concentrations of daily air pollutant on daily counts of emergency hospital admissions in 12 major hospitals. The effects of time trend, season, and other cyclical factors, temperature, and humidity were accounted for. Autocorrelation and overdispersion were corrected. Daily concentrations of nitrogen dioxide (NO_2), sulphur dioxide (SO_2), ozone (O_3), and particulate matter <10 μm in aerodynamic diameter (PM_{10}) were obtained from seven air monitoring stations in Hong Kong in 1994 and 1995. Relative risks (RR) of respiratory and cardiovascular disease admissions (for an increase of 10 μg/m³ in concentration of air pollutant) were calculated.**

***Results*—Significant associations were found between hospital admissions for all respiratory diseases, all cardiovascular diseases, chronic obstructive pulmonary diseases, and heart failure and the concentrations of all four pollutants. Admissions for asthma, pneumonia, and influenza were significantly associated with NO_2, O_3 and PM_{10}. Relative risk (RR) for admissions for respiratory disease for the four pollutants ranged from 1.013 (for SO_2) to 1.022 (for O_3), and for admissions for cardiovascular disease, from 1.006 (for PM_{10}) to 1.016 (for SO_2). Those aged ≥65 years were at higher risk. Significant positive interactions were detected between NO_2, O_3, and PM_{10}, and between O_3 and winter months.**

***Conclusions*—Adverse health effects are evident at current ambient concentrations of air pollutants. Further reduction in air pollution is necessary to protect the health of the community, especially that of the high risk group.**

(*Occup Environ Med* 1999;56:679–683)

Keywords: air pollution; respiratory diseases; cardiovascular diseases

In recent years, time series studies have been used extensively in the study of air pollution and health outcomes.[1–25] Positive associations between individual air pollutants and mortalities or morbidities have been found in many American and European studies.[1,8,10,30] Pooled estimates in 12 European cities of increase in daily mortality for an increase of 50 μg/m³ in concentrations of sulphur dioxide (SO_2) and particulates were 3% and 2% respectively.[8] Few time series studies have been reported in Asian cities except Beijing.[7] Hong Kong is a densely populated city in Southern China with hot, humid summers and mild, dry winters. Motor vehicles are the main source of air pollutants. In 1994 and 1995, the mean daily concentrations of nitrogen dioxide (NO_2) and particulates <10 μm in aerodynamic diameter (PM_{10}) were quite high, at 53.7 μg/m³ and 50.1 μg/m³ respectively.[31] Compared with western European cities, Hong Kong had a low concentration of SO_2 (daily mean 20.2 μg/m³), whereas ozone (O_3) concentrations were comparable (8 *hour mean* 28.7 μg/m³).[11,13,19,31] To elucidate the association between air pollutants and acute health effects, we performed a time series study on daily hospital admissions from 12 major hospitals and air pollutant concentrations from 7 air quality monitoring stations. The aim of the study was to examine the relation between concentrations of air pollutants and health effects from local data.

Materials and methods

HOSPITAL DATA

Emergency hospital admissions for respiratory and cardiovascular diseases in all 12 major hospitals for 1994 and 1995 were collected. A computerised format of patient data captured age, date of admission, and diagnosis on discharge from the ninth revision of the international classification of diseases (ICD-9).[32] Two groups of diseases were chosen: diseases of the respiratory system (ICD 460–466, 471–478, 480–487, and 490–496) and diseases of the cardiovascular system (ICD 410–417, 420–438, and 440–444). Also, the following diseases were analysed separately: asthma (ICD 493), chronic obstructive pulmo-

Table 1 Mean summary statistics of daily pollutant concentrations (μg/m³), meteorological variables, and hospital admissions, 1994–5

	Min	*25th Percentile*	*Median*	*75th Percentile*	*Max*
NO_2	16.41	39.93	51.39	66.50	122.44
O_3 (8 h)	0	11.82	23.15	43.35	129.94
SO_2	2.74	12.45	17.05	25.11	68.49
PM_{10}	14.77	30.66	44.99	65.45	159.71
Temperature (°C)	9.34	18.40	24.00	27.15	30.96
Humidity (%)	32.33	73.41	80.14	85.00	96.33
Respiratory admissions	84	116.75	131	150	232
Cardiovascular admissions	54	87	101	116	177