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DRAFT

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## COMMITTEE ON CARCINOGENICITY OF CHEMICALS IN FOOD CONSUMER PRODUCTS AND THE ENVIRONMENT

### OLFACTORY NEUROBLASTOMA: EVIDENCE FOR AN ELEVATED INCIDENCE AMONG DENTISTS? AN INITIAL DISCUSSION PAPER.

#### Introduction to Olfactory Neuroblastoma

1. Olfactory neuroblastoma (ONB. The alternative name is esthesioneuroepithelioma) is estimated to comprise approximately 3% of nasal neoplasms excluding benign polyps. The incidence in N. America/Western Europe is 0.15/million/year. There is no evidence for a sex difference in incidence. It occurs in all ages (but is rare below 10 y and over 70 y).<sup>1</sup> It has been reported to have bimodal incidence, with peaks in the 2<sup>nd</sup>-3<sup>rd</sup> decade and later in the 6<sup>th</sup> and 7<sup>th</sup> decades of life.<sup>2</sup> Broich and colleagues attempted to review all published cases since 1924, when ONB was first cited in the literature, up to 1997 and estimated there were only 950 cases cited in the scientific literature.<sup>3</sup> Thus the available evidence suggests that ONB is a very rare tumour. A number of background references are provided in Annex 1.
2. ONB is described as a neuroectodermal neoplasm showing predominantly neural features.<sup>4</sup> The most common symptoms in patients presenting with ONB are nasal obstruction (93%), epistaxis (55%) and rhinorrhea (30%). Other symptoms such as headache and anosmia occur at an incidence of below 10%.<sup>2</sup> Diagnoses is based on clinical presentation, CT/MRI screening and histology with the need for a battery of immunohistochemical stains to differentiate from other closely related head and neck cancers. Professor Pavel Dulgurov (from Department of Clinical Neurosciences, University of Geneva Medical School.) considers that tumours usually stain positive for S-100 protein and/or neuron-specific enolase and stain negatively for UMB 45 (distinguishing from melanoma), cytokeratin (distinguishing from sinonasal undifferentiating carcinoma) and myc-2 (distinguishing from Ewing sarcoma) <http://www.emedicine.com/med/topic748.htm>. One other group has presented evidence that S-100 protein is expressed in only a small proportion of cases.<sup>5</sup> Recently trkA and p75 neurotrophin receptor proteins were expressed in all 10 cases reviewed by one group of authors.<sup>6</sup> In some instances use of electron microscopy has been used for a definitive diagnosis.
3. Recently it has been demonstrated that ONB may exhibit selectively increased uptake of Technetium 99m-ethyl cysteinate dimer with visualisation by CT.<sup>7</sup> A number of tumour staging systems have been proposed. The most common currently used is based on the classical Tumour, Node, Metastasis (TNM) system. Grading is helpful in assessing the clinical course of ONB.

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## Case-report of ONB: Woodworker

4. The only published case-report of ONB where an occupational exposure aetiology has been suggested refers to a woodworker exposed to wood dust for 25 years.<sup>7</sup> COC members will be aware that IARC classified exposure to wood dust as group 1 (i.e. a known human carcinogen).

## Evidence for ONB in experimental animals

5. A brief search of the US National Library of medicine internet site (Chemical Carcinogenesis Research Information System, <http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?CCRIS>) identified a number of chemicals where olfactory neuroblastoma or esthesioneuroepithelioma were documented. From the limited information available it appears most of these chemicals could be considered as potential genotoxic carcinogens. Very limited information on the histology of tumours is available. Peter Magee noted a number of comments in his chapter from Searle's ACS Monograph 173 on Chemical Carcinogenesis on N-nitroso compounds. This is a very old text published in 1976 but considered to be authoritative at the time. Magee noted that the nasal cavities of rodents were particularly susceptible to the organotropism noted with N-nitroso compounds. In particular N-nitrosodimethylamine only induced tumours of the posterior region of the nasal cavity in hamster (esthesioneuroepitheliomas) whereas N-nitrosodiethylamine produced tumours in all parts of the nasal cavity. A brief tabulation of the data from the CCRIS is given below:

Chemical	ONB or esthesioneuroepithelioma	Other tumours?	Mutagenicity and other data
Procarbazine hydrochloride	ONB; male and female rats and mice. Studies used intraperitoneal dosing	Mice; uterine carcinoma, leukaemia, lymphoma, lung adenoma. Rat; lymphoma and mammary gland adenoma	<i>In-vitro</i> and <i>in-vivo</i> mutagen. IARC Group 2A.
p-cresidine	ONB; male and female F344 rats in a dietary long term bioassay (104 weeks)	Hepatic tumours (HCC and cholangiocarcinoma) urinary bladder tumours (including carcinoma and Transitional cell papilloma/carcinoma)	<i>In-vitro</i> mutagen in Salmonella typhimurium TA 100, 98 in presence of S-9 (rat/hamster) and in E.coli WP UVRA +S-9 (rat, hamster)
Azoxyethane	ONB: Rat (sex not given) subcutaneous.	Mammary gland, leukaemia, and glioma.	Alkylating agent. No mutagenicity data retrieved.
Bis-(chloromethyl)ether	Esthesioneuroepithelioma inhalation rat in two separate studies	Lung tumours	Mutagenic effects reported in workers exposed to BCME. IARC Group 1.
N-nitrosodimethyl	Esthesioneuroepithelioma male and female rats in	Spinal cord neuroma (male)	IARC Group 2A

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amine	gavage study (1/wk for life-time)		
N-nitroso-4-piperidine	Esthesioneuroepithelioma female rats in drinking water study (30 week exposure period)	Forestomach, oesophagus, tongue, .liver	No other data identified
3-methyl (nitrosoamino)p ropionitrile	Esthesioneuroepithelioma Female rats given subcutaneous doses 3/week for 20 weeks	Oesophagus, tongue, nasal.	No other data identified

### Histological classification of ONB

6. There are differences in the published literature with regard to nomenclature of ONB in humans. Some groups prefer the term esthesioneuroepithelioma arguing that the tumour is not a true blastoma. Other groups argue that the term “esthesio” refers to effects on sensory perception which isn’t appropriate for ONB. ICD 0 version 2 recognises three distinct histological classifications ICD 02 codes Esthesioneurocytoma 9521/3, Esthiobeuroblastoma 9522/3, Esthesioneuroepithelioma 9523/3 for ONB. The COC secretariat consulted the medical pathologist for COC and throughout this paper ONB has been considered as a single tumour with a wide variety of histological presentation. Members may wish to note that consideration as three separate tumours would infer a diversity of aetiological factors for each tumour type.

### Suggested hypothesis: ONB is associated with occupation as dental worker?

7. Professor Lund has reviewed data from 1300 patients presenting with tumours of the nose and paranasal sinus collected over the past 25 years by her group based at the Institute of Laryngology and Otolaryngology, University College London. She noted 4 out of the 50 cases of olfactory neuroblastoma had been employed at some time either as dentists or dental nurses (Annex 2). The data given in Annex 2 should be regarded as IN CONFIDENCE medical information. Precise details of exposure histories are not available at the time of writing. It is hoped Professor Lund will be able to provide more information at the 6 November 2003 COC meeting.
8. The secretariat were copied correspondence from Mr Lowry (consultant oral and maxillofacial surgeon) which identified potential exposure to oil of cloves (in particular eugenol) and to amalgam (in particular to mercury) as potentially relevant chemical exposures. (Annex 3)

### Suggested risk factors: Dental workers

9. The focus of this paper is on the consideration of the case-data provided by Professor Lund. If the committee considers there is

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sufficient information to suggest a hypothesis that chemical exposure might be involved in the aetiology of the ONBs, then it would be appropriate to undertake thorough reviews.

10. For the purposes of this initial discussion paper, very brief overviews of evidence regarding exposure of dental workers to potential carcinogens and information on the potential aetiological chemicals suggested in the correspondence copied to the secretariat (Annex 3) have been provided. The secretariat would undertake more thorough reviews as directed by members if the COC consider this is appropriate.

### Information pertaining to occupational exposures of dental workers.

11. A brief literature review was undertaken. An inventory of potential chemical exposures of dental workers has not been undertaken. It was noted from Hunters' Diseases of Occupations that there were published references to cases of dental technicians pneumoconiosis.<sup>8</sup> In one case report a lung biopsy had been undertaken and evidence for exposure to chromium, cobalt, silica reported.<sup>9</sup> A further literature review was undertaken to ascertain whether any publications had focused on adverse effects of chemical exposures on the nasal tissue in dental workers.
12. Burgaz and colleagues from the Gaza University, Ankara, Turkey undertook a micronucleus assay of exfoliated nasal epidermal cells using the cytokinesis-block assay (CB-MN)<sup>10</sup> (Annex 4). They studied 27 dental laboratory technicians and 15 control subjects. The differences in the urinary excretion of metals (chromium, cobalt and nickel) between technicians and controls were statistically significant. The mean (+/-S.D.) CB-MN frequencies (per thousand ) in peripheral lymphocytes were 4.00 (+/-2.98) among the dental technicians and 1.40 (+/-1.30) among the controls, a statistically significant difference ( $P < 0.005$ ). The mean (+/-S.D.) MN frequencies (per thousand ) in nasal cells were 3.50 (+/-1.80) among the dental technicians and 1.19 (+/-0.53) among the controls, which was also a statistically significant difference ( $P < 0.005$ ). There was a significant correlation between duration of exposure and MN frequencies in lymphocytes ( $r = 0.642$ ,  $P < 0.01$ ), but not in nasal cells of technicians. The authors attempted to adjust for smoking as an important confounding factor. The authors suggested that *in vivo* exposure to chromium, nickel and cobalt metals is evident and that this occupational exposure may contribute to the observed genotoxic damage in two types of cells, e.g. lymphocytes and exfoliated nasal cells. However, it was not possible to determine which compound(s) were responsible for the genotoxic damage observed in this study.
13. Thus overall there is evidence that certain dental workers (technicians involved in production of crowns, bridges etc) may be occupationally exposed to mutagenic chemicals. No conclusions have been

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suggested on whether the exposures cited by Burgaz are relevant to the dental workers with ONB.

### Information on chemicals suggested as potentially relevant.

14. Mr Lowry (consultant oral and maxillofacial surgeon) suggested that exposure to oil of cloves (specifically citing eugenol) and to amalgam/mercury might be relevant to the aetiology of ONB (Annex 3).  
*Oil of Cloves:* Cloves contain up to 20% volatile oil, gallic acid, crystalline principles (caryophyllin and eugenin), gum, resin, fibre.  
**Actions:** stimulant, carminative, aromatic, anodyne, antiemetic, antiseptic.  
**Indications:** nausea, vomiting and flatulence.  
**Therapeutics and Pharmacology:** A few drops of the oil in water will stop vomiting and an infusion will relieve nausea. Eugenia (oil of cloves) is a powerful local antiseptic and mild anaesthetic which may be used topically in toothache. For toothache, put a clove near the tooth and keep in the mouth, or use clove oil on a little cotton wool.
15. Eugenol (a constituent of oil of cloves) is listed by IARC as Group 3 (i.e. chemical is not classifiable as to its carcinogenicity in humans). (Annex 5) IARC noted limited evidence for carcinogenicity in mice. The IARC evaluation has not been updated since 1987. <http://www-ie.iarc.fr/htdocs/monographs/vol36/eugenol.html>
16. The IARC evaluation is based predominantly on U.S. National Toxicology Program (NTP) carcinogenicity bioassays conducted in 1983. Eugenol was given in the diets of female F344/N rats (0, 0.6, or 1.25%) and of male F344/N rats and male and female B6C3F1 mice (0, 0.3, or 0.6%) for 103 weeks. Under these experimental conditions, there was no evidence of carcinogenicity observed for male or female rats. For mice there was equivocal evidence of carcinogenicity since eugenol caused increased incidences of both carcinomas and adenomas of the liver in male mice at the 3,000 ppm dietary level and because eugenol was associated with an increase in the combined incidences of hepatocellular carcinomas or adenomas in female mice.
17. There are some additional mutagenicity data on eugenol published since the IARC review (see Annex 5).<sup>11,12</sup> A full review has not been undertaken for this initial discussion paper. A positive response has been claimed in a bone-marrow micronucleus test in mice using intraperitoneal administration of eugenol. In a separate study eugenol was not mutagenic in the liver in a study using *LacZ* transgenic mice. However the authors documented evidence for formation of DNA adducts in *Lac Z* transgenic mice. The discussion sections of these two papers identify a conflicting literature on the mutagenicity of eugenol. Overall the evaluation of the mutagenicity of eugenol would be complex and it might be difficult to reach any conclusions. No conclusions are suggested in this paper.

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18. *Amalgam/Mercury*. IARC concluded that metallic mercury should be included in group 3 (i.e. chemical is not classifiable as to its carcinogenicity in humans.) in 1993. <http://www-cie.iarc.fr/htdocs/monographs/vol58/mono58-3.htm> . (Annex 6) The available but limited epidemiology information from two case-control studies suggested conflicting results with a small increased risk of brain tumours in one report and no evidence in a separate report. The information on cytogenetic studies of occupational exposure to metallic mercury also gave conflicting results. Overall there does not seem to be any compelling evidence to suggest that exposure to metallic mercury is associated with cancer.

### COC Discussion

19. The Committee has been asked to advise as to whether the cases of ONB identified by Professor Lund might be due to occupational exposure to carcinogens. No definite conclusions have been suggested in this paper until COC members have been able to evaluate the information on the cases of ONB and in particular the exposure history information. ONB is a very rare tumour diagnosis. Only one case report was identified where potential occupational exposure to wood dust might have been involved in the aetiology of ONB. Four cases of ONB have been identified where the individual worked for some time as a dentist or dental nurse. It is noteworthy that no case of ONB among dental technicians was identified. A key question for COC members is whether the identification of four cases where employment as a dentist or dental nurse is sufficient evidence for a detailed review. Evidence that dental workers (technicians involved in producing crowns, bridges) are exposed to potential mutagens can be identified from the published literature. However it is not possible to link the exposures in these published data to the cases of ONB identified by Professor Lund since these cases refer to dentists and dental nurses. (The secretariat is attempting to obtain further information on chemical exposure of dentists, dental nurses and dental technicians). This subject was raised by Mr Lowry in his letter. A number of potential exposures of dentists and other dental workers to chemicals have been suggested as being potentially relevant to ONB. The available information on these chemicals (eugenol and mercury) has been reviewed very briefly. The data suggest that the evaluation of eugenol would be complex and it might be difficult to reach conclusions. No conclusions are suggested in this paper. The evidence for amalgam/metallic mercury are conflicting, but overall there is no convincing evidence that mercury is carcinogenic to humans.
20. It is noteworthy that this paper has concentrated on the chemical exposures suggested in correspondence. However the potential range of chemical exposures of dental workers would vary with job title/duties and over time and are thus likely to be very complex. Thus for example, there is published evidence that certain zinc-oxide eugenol based or resin-based root canal sealers can give positive results in *in-*

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*in vitro* mutagenicity tests and COMET assays for DNA damage.<sup>13,14</sup> Thus if COC considered there was sufficient evidence to warrant further work, there is a question as to how relevant chemical exposures could be identified.

21. One further suggestion was exposure to methylmethacrylate. This is the base material that dentures are made from. Inhalation exposure may occur when dentures are eased or adjusted. An US Environmental Protection Agency review of methylmethacrylate (MMA) published in 1998 and produced as part of the Integrated Risk Information System (IRIS) was consulted. <http://www.china-pops.net/enwww/IRIS-Mirror/subst/1000.htm#II>. The available information indicated that there is no convincing evidence for carcinogenicity in workers occupationally exposed to MMA. There is no evidence for carcinogenicity from long term inhalation carcinogenicity bioassays in rats, mice and hamsters. There is evidence to suggest that MMA is a severe nasal irritant affecting the olfactory epithelium and inducing inflammatory and degeneration of the epithelium. MMA is positive in several *in-vitro* mutagenicity tests but all available *in-vivo* mutagenicity tests are negative. This information suggests that MMA is unlikely to be involved in any nasal tumours seen in dentists.
  
22. The Committee is invited to consider the following suggested discussion points.
  - A. It is not possible to draw any conclusions from these limited data as to whether occupational exposure of dentists and dental nurses is associated with Olfactory neuroblastoma on the basis of the submitted data.
  
  - B. Is the identification of four cases of this rare tumour sufficient to generate a hypothesis that occupational chemical exposure may be important in the aetiology of these tumours and warrants further investigation?
  
  - C. If this is the case, should work concentrate on investigating exposure of dental workers to potential carcinogens?. Which groups of dental workers should be included?

**Secretariat October 2003**

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## References

1. Henk JM (1996). Uncommon tumours of the head and neck region Chapter 6.11 in volume 1 of the Oxford textbook of Oncology, edited by Peckham M, Pinedo H and Veronesi U. Oxford University Press, Oxford, pp1059-1064.
2. Lund VJ et al (2003). Olfactory neuroblastoma: Past, present and future. The Laryngoscope, 113, 502-507.
3. Brioch G, Paglairi A and Ottaviani F (1997). Estesioneuoblastoma: A general review of the cases published since the discovery of the tumour in 1924. Anticancer Research, 17, 2683-2706.
4. Mill SE (2002). Neuroectodermal neoplasms of the head and neck with emphasis on neuroendocrine carcinomas. Modern pathology, 15, 264-278.
5. Lund VJ and Milroy C (1993). Olfactory neuroblastoma: Clinical and pathological aspects. Rhinology, 31, 1-6.
6. Zhao SP and Zhou XF (2002). Co-expression of trkA and p75 neurotrophin receptor in extracranial olfactory neuroblastoma cells. Neuropathology and Applied neurobiology, volume 28, 301-307.
7. Magnavita N et al (2003). Aesthesioneuroblastoma in a woodworker. Occupational Medicine, 53, 231-234.
8. Prado GL et al (2001). Olfactory Neuroblastoma visualised by Technetium <sup>99m</sup>Tc-ECD SPECT. Radiation Medicine, 19, 267-270.
9. Burgaz S et al (2002). Assessment of cytogenetic damage in lymphocytes and in exfoliated nasal cells of dental laboratory technicians exposed to chromium, cobalt and nickel. Mutation Research, 521, 47-56
10. Ellahuene MF et al (1994). Genotoxic evaluation of eugenol using the bone marrow micronucleus assay. Mutation Research, 320, 175-180.
11. Rombelberg CJM et al (1996). Effect of eugenol on the mutagenicity of benzo(a)pyrene and the formation of benzo(a)pyrene-DNA adducts in the  $\lambda$ -*lacZ*-transgenic mouse. Mutation Research, 369, 87-96.
12. Tai K-W et al (2002). A Assessment of the genotoxicity of resin and zinc-oxide eugenol-based root canal sealers using an in vitro mammalian test system. J Biomed Mater Res. Jan;59(1):73-7.
13. Huang TH, Lee H and Koa CT (2001). Evaluation of the genotoxicity of zinc oxide eugenol-based, calcium hydroxide-based, and epoxy resin-based root canal sealers by comet assay. J Endod. 2001 Dec;27(12):744-748.