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ABSTRACT

Organic dyes have found increasing use as sensitizers in laser surgical procedures, due to their high optical absorbances. Little is known, however, about the nature of the degradation products formed when these dyes are irradiated with a laser. Previous work in our laboratories has shown that irradiation of polymeric and biological tissues with CO_2 and Nd:YAG lasers produces a host of volatile and semivolatile by-products, some of which are known to be potential carcinogens. This work focuses on the identification of the chemical by-products formed by diode laser (805 nm) and Nd:YAG (1.06 μm) laser irradiation of indocyanine green (ICG) and carbon black based ink sensitized tissues, including bone, tendon and sheep's teeth. Samples were mounted in a 0.5-L Pyrex sample chamber equipped with quartz optical windows, charcoal filtered air inlet and an outlet attached to an appropriate sample trap and a constant flow pump. By-products were analyzed by GC/MS and HPLC. Volatiles identified included benzene and formaldehyde. Semivolatiles included traces of polycyclic aromatics, arising from the biological matrix and inks, as well as fragments of ICG and the carbon ink components. The significance of these results will be discussed, including the necessity of using appropriate evacuation devices when utilizing lasers for surgical procedures.

Keywords: indocyanine green, carbon black, dyes, laser, chemical by-products, benzene, formaldehyde

1. INTRODUCTION

The use of lasers for surgical applications has found broad acceptance, due to its many advantages, including precision and speed. However, there are also potential disadvantages to using lasers for surgery, including ocular damage, the possibility of bacterial or viral transmission through the plume, and the generation of hazardous chemical products during the laser initiated decomposition of tissue.[1-5] Ocular damage has been minimized through the use of appropriate light filtering goggles and viral/bacterial transmission has been managed through the use of smoke evacuation devices. Chemical by-product hazards , evidenced by the smell associated with laser tissue cutting, have also been reduced through the use of efficient smoke evacuation devices. Thus, most of the potential hazards to operating personnel have been eliminated or reduced through the use of appropriate safety measures. The question of potential harm to patients by the formation of hazardous chemicals is still to be resolved, however.

Ott, for instance, has shown that smoke formed during laser surgery within the peritoneal cavity, is quickly absorbed into the patient's bloodstream, causing elevated methaemoglobin levels.[6] There is one additional practical problem associated with the use of lasers: the efficiency of laser light absorption. Lasers produce light in various regions of the spectrum, ranging from the ultraviolet (excimer lasers, 180 - 350 nm) to the near infrared (Nd:YAG, 1.06 μm) to the mid infrared (CO_2, 10.6 μm). Unfortunately, no single laser wavelength is suitable for all biological tissues, due to varying water content, chemical make-up and the susceptibility to thermal damage.[1] One method, becoming increasingly popular for enhancing the absorption of the laser energy, is to apply a dye to the tissue surface. The dye chosen has a maximal absorption of light close to the operating wavelength of the laser. Thus, reduced laser energy operating levels are possible, with increased ablation efficiencies while reducing potential hazards from the laser beam and decomposition products. Indocyanine green (ICG) is one dye which has been used successfully as a photoenhancer for tissue welding.[7,8] ICG seems well suited for microsurgical procedures, since it possesses low toxicity and high optical absorbance at ~800 nm, where most biological tissues are relatively transparent. However, little is known about the degradation products of ICG, and their potential toxicity, when exposed to laser light.[9,10] The current study resulted from a desire to use ICG and a carbon black based dye as photoenhancers in microsurgical procedures utilizing a diode laser (805 nm) and a Nd:YAG laser (1.06 μm). The relative hazard, due to chemical by-product formation, posed by the interaction of the laser light with these dyes and the tissue was unknown. A study was therefore undertaken to determine the amounts of representative hazardous chemicals present in the resulting smoke plumes produced when dye enhanced tissues were irradiated.

2. MATERIALS AND METHODS

2.1 Tissue samples

The laser surgical procedures to be studied included cutting of the bones of the middle ear, ablation of spinal discal material, and removal of superficial dental caries. The middle ear bone was represented in this study by a chicken bone, coated with a dried 3% solution of ICG and cut with a Surgimedics Diomed 805 nm diode laser. The spinal discal material was represented by a similarly ICG coated pig tendon, again cut with the diode laser. The dental material was represented by sheep's teeth, coated with a proprietary carbon black based injet formulation dye and cut with an Americal Dental Technologies, Incisive Technologies Division Pulse Maser 1000U Nd:YAG medical laser (1.06 μm). In addition, reference samples were prepared by coating standard microscope slides with thin coatings of the ICG and carbon inks.

2.2 By-product collection

The samples were weighed immediately before and after laser cutting. Each sample was mounted in a 500-cc cylindrical Pyrex chamber (Figure 1) equipped with a quartz window, an activated carbon filtered air inlet (SKC Inc. charcoal sampling tube), and a sample collection tube. Air was drawn from the chamber into the collection tube at a constant velocity using a calibrated sampling pump (SKC Inc.). Volatile and semivolatile chemical by-products were first collected on activated charcoal tubes and ORBO-43 tubes (Supelco, Inc.), respectively. These tubes were then solvent extracted (CS_2) and analyzed by gas chromatography/mass spectroscopy (GC/MS). The solvent extraction technique, however, was not found to be sensitive enough and was subsequently replaced with an adsorption/thermal desorption tube (200 cc/minute collection) for analysis of both volatile and non-volatile components. Aldehydes were analyzed by collection on Supelco dinitrophenylhydrazine (DNPH) coated silica gel tubes at a sampling rate of 1 L/minute.

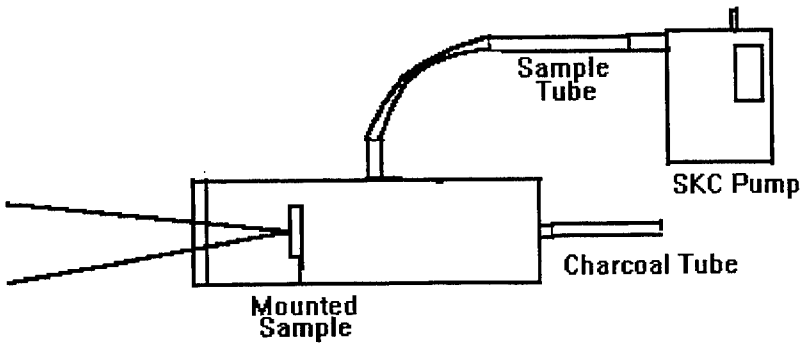

Figure 1. Sampling chamber used for sample irradiation and by-product collection

2.3 Laser operating parameters

The optic fibers for the diode and Nd:YAG lasers (400 μm diameter) had laser beam divergences such that it was necessary to use a quartz double condensing lens system (Figure 2) to focus the beam within the chamber. Actual spot size on the sample was 800 μm, decreasing the laser power density by 75%. Also decreasing laser power density was a 3% light reflection loss at each lens and window surface. The Nd:YAG laser was operated in the pulsed mode, 280 mJ at 25 Hz with approximately 5 W delivered to the sample. The diode laser was also operated in the pulsed mode, at a 0.5 second duration and a 0.1 second interval between pulses with ~ 6 W delivered to the sample. The sample chamber was mounted on an XY table and the sample then moved through the beam at a rate of 0.22"/second.

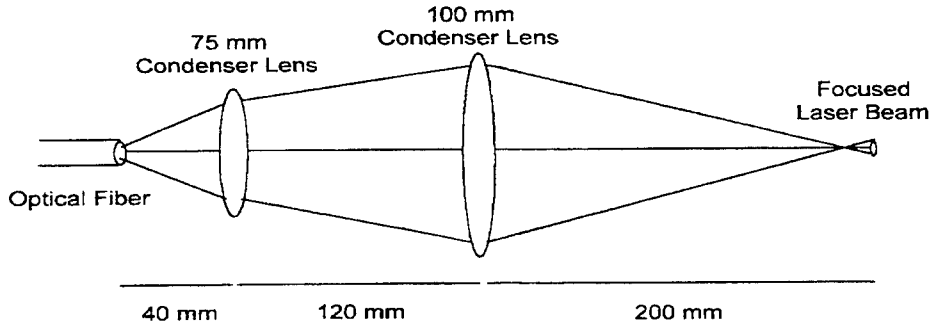

Figure 2. Condensing lens used to focus laser beam from the optical fiber onto the sample in the chamber

2.4 Aldehyde analyses

Aldehyde by-products were collected on Supelco DNPH sample tubes and analyzed by high performance liquid chromatography (HPLC). Two blanks were obtained for each set of samples collected by drawing an equivalent volume of air through the chamber onto the tube, with a sample mounted in the chamber. Each DNPH tube was eluted with 5.0 mL of HPLC grade acetonitrile into a 5-mL volumetric flask, and a portion subjected to HPLC analysis. The HPLC instrument used was a Waters 616 system with a Waters 996 photodiode array detector set at 360 nm and a 717 autosampler. The separation column was a Waters Nova-Pak C18 (150 mm x 3.9 mm, 4 μm diameter packing). Themobile phase flow was 1.0 mL/minute. Solvent A was acetonitrile : tetrahydrofuran : water, 30 : 10 : 60; solvent B, acetonitrile : water, 60 : 40. The gradient program used was 0 % B for 1 minute, followed by a linear gradient to 100% B over 10 minutes, and held at 100% B for 15 minutes. Under these conditions, separations were obtained for all but two aldehyde/ketone DNPH derivative standards (Supelco, Inc.): methacrolein and 2-butanone (Figure 3). Retention times for each standard are given in Table 1.

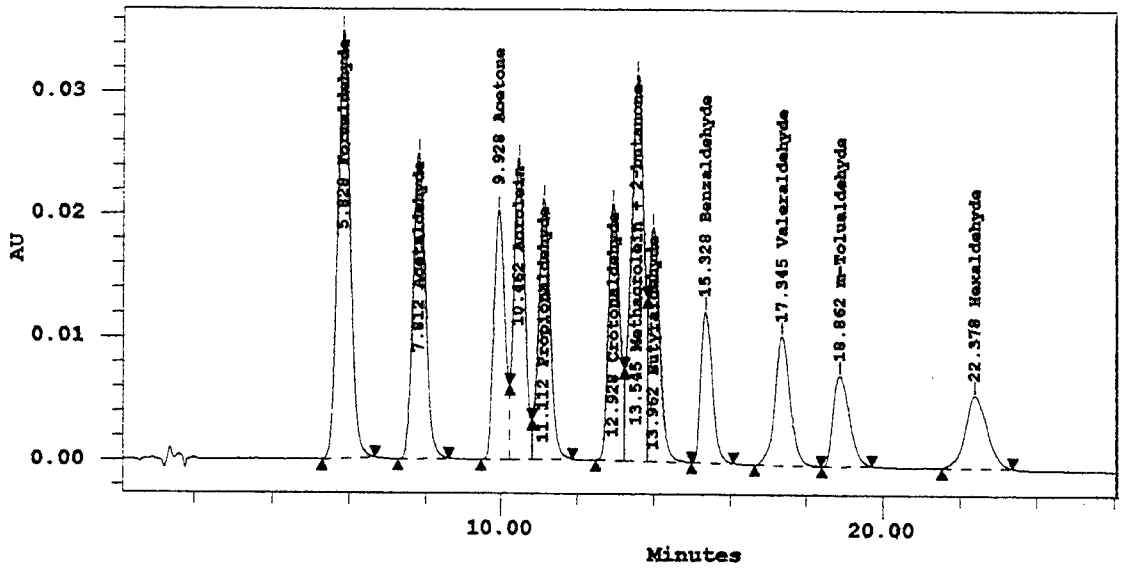

Figure 3. HPLC separation of aldehyde and ketone DNPH derivatives

Aldehyde/Ketone	HPLC Retention Time (min)
Formaldehyde	5.828
Acetaldehyde	7.812
Acetone	9.928
Acrolein	10.462
Propionaldehyde	11.112
Crotonaldehyde	12.928
Methacrolein + 2-Butanone	13.545
Butyraldehyde	13.962
Benzaldehyde	15.328
Valeraldehyde	17.345
m-Tolualdehyde	18.862
Hexaldehyde	22.378

Table 1. HPLC retention times for aldehyde/ketone DNPH standards in Figure 3

2.5 Volatile/semivolatile hydrocarbon analyses

Analysis for volatile and semivolatile hydrocarbons used a modification of the National Institute of Occupational Safety and Health (NIOSH) method 2549.[11] This involved trapping the by-products in a 4 inch x 1/4 inch diameter stainless steel adsorption tube (inner surface coated with Silcosteel, Restek, Inc) consisting of a three-layer adsorbent medium: Carbotrap C (300 mg), Carbotrap (200 mg) and Carbosieve S-III (125 mg) (Supelco, Inc). Two blanks were collected for each set of samples. Each tube was spiked with 1 µL of a methanol solution containing 181 ng of decafluorobiphenyl, which acted as an internal standard. Quantitative standards were obtained for benzene, toluene, ethylbenzene, o-, m, p-xylene (BTEX standards, Supelco, Inc) by injecting 1 µL methanol standards onto the trap. Semivolatile polycyclic aromatic hydrocarbon standards were also run separately to obtain their respective gas chromatographic (GC) retention times. The tube was mounted in a Tekmar 4000 purge and trap (PNT) device, modified to allow thermal desorption at 300 °C and interface line operation at 200 °C. Sample

tubes were purged for 1 minute with helium to remove air and methanol and then thermally desorbed at 20 cc/minute at 300 °C onto a Hewlett-Packard 5990 Series II GC interfaced to a HP - 5971A benchtop mass spectrometer (MS). The GC was operated with the split-splitless inlet purge on, 20 cc/minute, and septum purge 2 cc/minute. The PNT transfer line was connected through the inlet septum and supplied 20 cc/minute, the GC inlet an additional 2 cc/minute. With a column flow rate of 0.5 cc/minute, the split ratio was therefore 40 : 1. The chromatography column used was a HP-5, 25 m x 0.2 mm i.d. with a 0.25 µm column coating. GC oven parameters were as follows:10 °C for 4 minutes, ramped at 6°C/minute to 100°C, held at 100°C for 4 minutes, then ramped at 20 °C to 300 °C and held at 300 °C for 1 minute. MS acquisition began 6 minutes after initiation of desorption. A typical BTEX analysis, with a concentration of 400 ng for each BTEX component and 181 ng for decafluorobiphenyl is shown in Figure 4. The components and retention times for the BTEX mixture are given in Table 2.

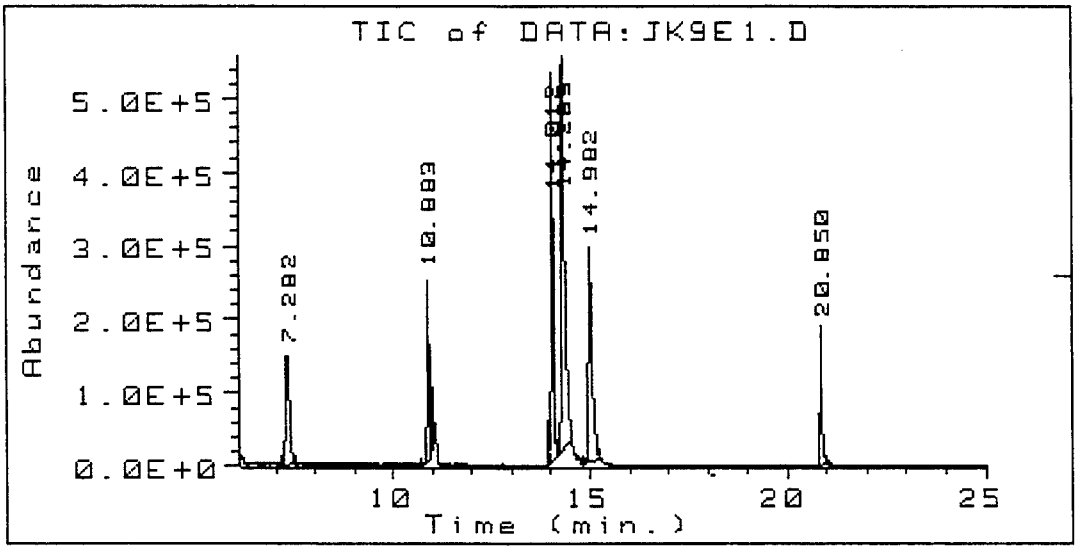

Figure 4. GC/MS total ion chromatogram for BTEX mix and decafluorobiphenyl

Component	Amount	Retention Time (min)
Benzene	400 ng	7.282
Toluene	400 ng	10.883
Ethylbenzene	400 ng	14.012
m-, p-Xylene	400 ng each	14.289
o-Xylene	400 ng	14.982
Decafluorobiphenyl	181 ng	20.850

Table 2. Components and retention times of BTEX and internal standard (GC/MS)

3. RESULTS

Previous analyses of laser generated by-products of biological samples indicated that a complex mixture of chemicals would be found in the samples studied in this work.[1,3] This was indeed the case. A typical example of a GC/MS trace for a carbon black ink coated tooth cut with the Nd:YAG laser is shown in Figure 5. Components include benzene, toluene, the xylenes and naphthalene (retention time 21.762 min). A typical HPLC trace for aldehydes and ketones obtained from a bone sample using the diode laser is shown in Figure 6.

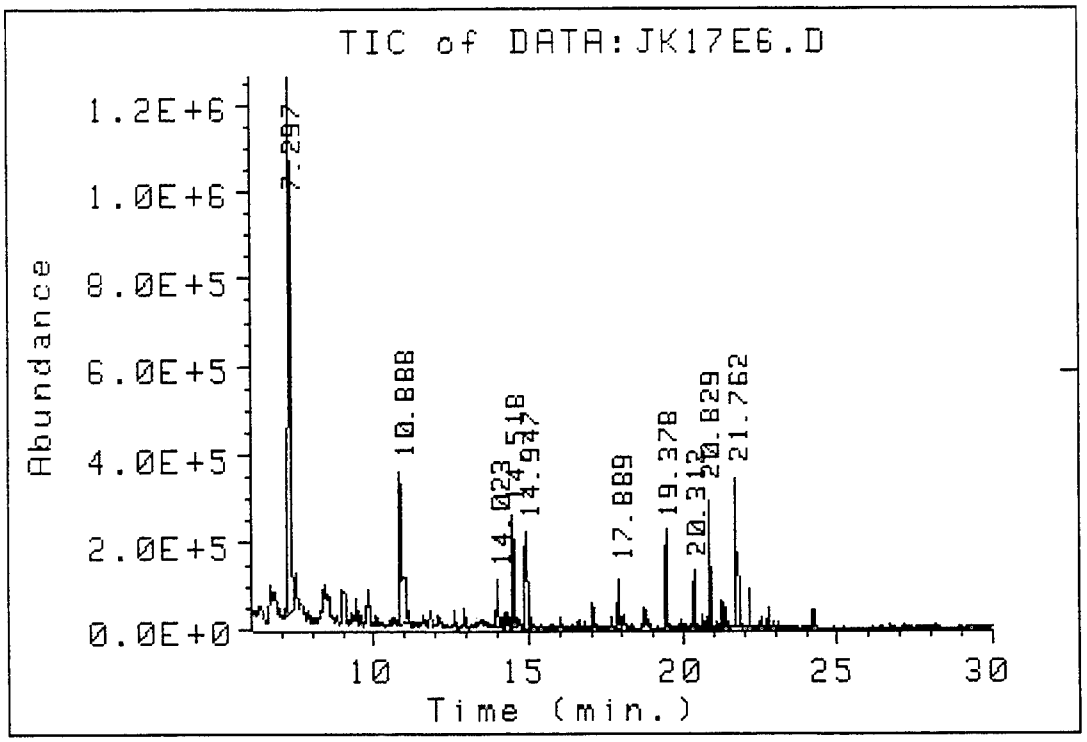

Figure 5. GC/MS chromatogram for hydrocarbon by-products obtained by Nd:YAG laser cutting a carbon black ink coated tooth

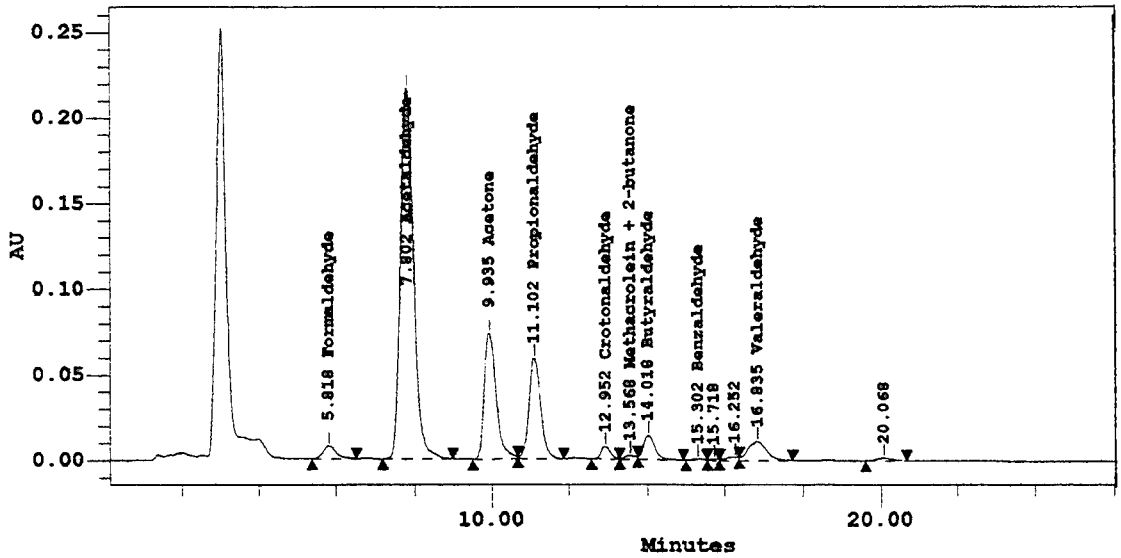

Figure 6. HPLC chromatogram for aldehyde and ketone DNPH derivatives obtained by diode laser cutting ICG coated bone

Table 3 summarizes the results obtained for the analyses of benzene and formaldehyde formed by cutting ICG and carbon ink coated slides with the diode and Nd:YAG lasers (average of two samples each), cutting bone and tendon samples coated with ICG and carbon ink with the diode laser (average of two samples each), and cutting teeth coated with carbon ink with the Nd:YAG laser (average of four samples).

Sample	Benzene ng/mg	(ppm/mg)	Formaldehyde µg/mg	(ppm/mg#)
slide/C*/D**	65	(0.02)	3.0	(2.4)
slide/I***/D	510	(0.16)	0.1	(0.08)
slide/C/N****	---	------	0.4	(0.4)
bone/C/D	572	(0.18)	3,6	(2.9)
bone/I/D	110	(0.03)	Not Detected	(Not Detected)
tendon/C/D	1.1	(5×10^{-4})	0.2	(0.2)
tendon/I/D	2.2	(3×10^{-5})	Not Detected	(Not Detected)
teeth	146	(0.04)	0.4	(0.32)

* carbon based ink, ** diode laser, ***ICG ink, **** Nd:YAG laser, # based on a volume of 1 L

Table 3. Average amounts of benzene and formaldehyde formed during cutting of tissues

For comparison, the complete listing of aromatic hydrocarbon and aldehyde/ketone compounds obtained from a tooth (GC/MS trace represented in Figure 5) and bone (HPLC trace in Figure 6) are listed in Tables 4 and 5.

Aromatic Hydrocarbon	GC/MS Retention Time (min)	Amount Detected (ng)
Benzene	7.297	1643
Toluene	10.890	319
Ethylbenzene	14.023	40
m-, p-Xylene	14.518	153
o-Xylene	14.947	170
Decafluorobiphenyl	20.829 (internal standard)	181

Table 4. Aromatic hydrocarbons obtained from one tooth sample (GC/MS trace Figure 5). Total material removed, 13.8 mg.

Aldehyde/Ketone	HPLC Retention Time (min)	Amount Detected (ng)
Formaldehyde	5.818	962
Acetaldehyde	7.802	39900
Acetone	9.935	17600
Acrolein	10.462	Not Detected
Propionaldehyde	11.102	12500
Crotonaldehyde	12.952	1240
Methacrolein + 2-Butanone	13.568	256
Butyraldehyde	14.018	3400
Benzaldehyde	15.302	277
Valeraldehyde	16.835	5610
m-Tolualdehyde	18.862	Not Detected
Hexaldehyde	22.378	Not Detected

Table 5. Aldehydes and ketones obtained from one bone sample (HPLC trace Figure 6). Total material removed, 5.2 mg.

4. DISCUSSION OF RESULTS

The significance of the results obtained in this study must be looked at in the context of the limitations of the experimental data and the relative hazards of the chemicals analyzed. The purpose of this study was to provide a measure of the potential hazards which laser generated decomposition products might pose to laser operating personnel and patients. However, conditions employed for laser cutting were meant to mimic, but not duplicate, conditions to be employed during actual surgical or dental procedures. In fact, actual surgical or dental laser operating conditions to be finally employed will be modified, if possible, to minimize by-product hazards. Thus, the laser systems will be operated at a minimum energy level, with good gas evacuation systems, to reduce the laser plume and by-product concentrations. In addition, the photoenhancing inks employed will be dispensed as measured microdrops, immediately before a laser pulse, decreasing the total amount of ink interacting with the laser beam.

With these differences between actual surgical/dental procedures and the experimental conditions utilized for this work, what is the significance of the types and amounts of the chemicals detected as health hazards for laser operating personnel and patients? Table 6 lists the NIOSH, OSHA and ACGIH permissible limits for the chemicals determined in this study.[11-13] Note that these limits pertain to allowed limits for the laser operating personnel only, and do not pertain to patients. In fact, there are no established exposure limits for patients, and such limits would be different for different operating procedures, if they did exist. Of the chemicals in this list, two deserve close attention, benzene and formaldehyde. Both have very low worker exposure limits, since they are suspect carcinogens. Both of these chemicals were found in measurable quantities in all of the smoke samples analyzed (Table 3), and this is also true for laser decomposition studies previously carried out by us.[1,3] The question is whether or not these quantities represent a true hazard, or are so small that they can be ignored. This depends on how one looks at the data. Atmospheric levels, measured in parts per million (ppm) or mg/m^3, are certainly lower than even the NIOSH limits, even if a smoke evacuator were not used, given the tiny amounts of material vaporized during a microsurgical or dental procedure, presuming the laser operator is not close enoough to the site to enhale the fumes directly. But, how about the patient? Given Ott's study of the effect of peritoneal cavity laser surgery, it may not be necessary for the patient to breath the vapors in order to suffer ill effects from them.[6] This leads to the conclusion that, despite the very small amount of material produced during these studies, a smoke evacuation system will be mandatory for these types of microsurgery or dental procedures, to protect the patient.

Chemical	OSHA Limits (ppm)	NIOSH (ppm)	ACGIH (ppm)
Formaldehyde	3; C* 5; P** 10/30 min	0.016; C 0.1, Carc***	C 0.3 Suspect Carc
Acetaldehyde	200	18, Carc	100 STEL****
Acrolein	0.1	0.1; 0.3 STEL	0.3 STEL
Crotonaldehyde	2	2	2
Valeraldehyde	---	50	50
Benzene	1; 5 STEL	0.1, C 1ppm/15 min, Suspect Carc	10, Suspect Carc
Toluene	200; C 300; P 500	100; STEL 150	50
Ethylbenzene	100	100, 125 STEL	100
o-, m-, p-Xylene	100	100; 150 STEL	100; 150 STEL

* Ceiling concentration, ** maximum permissible, instantaneous, *** Carcinogen, ****short-term (15 min) exposure limit

Table 6. Permissible hazardous chemical atmospheric limits allowed for workers

5. CONCLUSIONS

Small quantities of potentially hazardous chemicals are formed when diode and Nd:YAG lasers are used, in conjunction with a photoenhancing dye, to cut biological materials. Results indicate that the photoenhancing dyes do not contribute significantly to the by-product concentration, compared to the products formed by decomposition of the biological materials. Use of an appropriate smoke evacuation device in conjunction with this laser ablation technique is mandatory, in order to protect the patient from potential harm by these by-products.

6. ACKNOWLEDGEMENTS

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7. REFERENCES

1. J. M. Kokosa and M. Benedetto, "Probing plume protection problems", *J. Laser App.*, Vol. 4, pp. 39-49, 1992.

2. M. S. Baggish, B. J. Poiecsz, D. Jaret, P. Williamson and A. Refei, " Presence of human immunodeficiency virus DNA in laser smoke", *Lasers Surg. Med.*, Vol. 11, pp. 197-203, 1991.

3. J. M. Kokosa and J. Eugene, "Chemical composition of laser-tissue interaction smoke plume", *J. Laser App.*, Vol. 1, pp. 59-63, 1989.

4. R. H. Clark, J. M. Ismer, R. F. Donaldson and G. Jones II, "Gas chromatographic-light microscopic analysis of excimer laser photoablation of cardiovascular surgery: ablation products and photoacoustic spectrum of cardiovascular wall", *Cir.. Res.*, Vol 60, pp. 429-437, 1987.

5. N. P. J. Walker, J. Mathews and S. W. B. Newsom, "Possible hazards from irradiation with the carbon dioxide laser", *Lasers Surg. Med.*, Vol. 6, pp. 84-86, 1986.

6. D. Ott, " Smoke production and smoke reduction in endoscopic surgery: preliminary report", *End. Surg.* Vol. 1, pp. 230-232, 1993.

7. S. D. DeCoste, W. Forinelli, T. Flotte and R. R. Anderson, "Dye-enhanced laser welding for skin closure", *Laser Surg. Med.*, Vol. 12, pp. 25-32, 1992.

8. M. C. Oz, J. P. Johnson, S. Paranzi, R. S. Chuck, C. C. Marboe, L. S. Bass, R. Nowygrod and M. R. Treat, "Tissue soldering by use of indocyanine green dye-enhanced fibrogen with the neear infrared diode laser", *J. Vasc. Surg.*, Vol. 11, pp. 718-725, 1990.

9. D. Dimikov, L. S. Bass and M. R. Treat, "Thermal breakdown properties of indocyanine green", *Proc. SPIE*, Vol. 2395, pp. 486-489, 1995.

10. J. F. Zhou, M. P. Chin and S. A. Schafer, "Aggregation and degradation of indocyanine green", *Proc. SPIE*, Vol. 2129, pp. 495-505, 1994.

11. *First supplement to NIOSH manual of analytical methods (NMAM)*, 4th Edn., 1996.

12. *Threshold limit values for chemical substances and physical agents and biological exposure indices for 1993-1994*, ACGIH, Cincinnatti, OH, 1993.

13. *OSHA analytical methods manual*, 2nd Edn. Part 1, Organic substances, Vol. 1-4, 1990.