

The release of biologically hazardous chemical compounds from orthodontic adhesive systems used in Europe and in the USA – an *in vitro* study

Konrad Małkiewicz^{1),*}, Piotr Wychowański²⁾, Elżbieta Małkiewicz³⁾,
Dorota Olczak-Kowalczyk⁴⁾, Jadwiga Turło⁵⁾

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Abstract: The study assessed the chemical stability of ConTec LC, Resilence, Light Bond and Transbond XT orthodontic adhesive resins. The subject of the analysis was the release of HEMA (2-hydroxyethylmethacrylate), TEGDMA (triethylene glycol dimethacrylate), EGDMA (ethylene glycol dimethacrylate), UDMA (urethane dimethacrylate) and BPA (bisphenol A) monomers from test sample materials in successive time intervals, using HPLC (high pressure liquid chromatography). The study confirmed the presence of methacrylate monomers and bisphenol A in eluates of the assessed materials regardless of the observation period. The results show that orthodontic adhesive systems polymerized with visible light are chemically unstable, releasing biologically harmful ingredients to the external environment for a long time. Dental materials containing polymer network based on methacrylates require structure modification or elimination of harmful components in order to increase safety of their application.

Keywords: orthodontic adhesive systems, monomers release, HPLC.

Uwalnianie biologicznie szkodliwych związków chemicznych z dostępnych na rynkach Europy i USA ortodontycznych systemów adhezyjnych – badania *in vitro*

Streszczenie: Oceniano stabilność chemiczną ortodontycznych żywic adhezyjnych ConTec LC, Resilence, Light Bond oraz Transbond XT. Metodą wysokociśnieniowej chromatografii cieczowej (HPLC) analizowano uwalnianie z próbek badanych materiałów monomerów: HEMA (metakrylan 2-hydroksyetylu), TEGDMA (dimetakrylan triglikolu etylenowego), EGDMA (dimetakrylan glikolu etylenowego), UDMA (dimetakrylan uretanu) i BPA (bisfenol A) w kolejnych przedziałach czasowych. Badania potwierdziły obecność monomerów metakrylanowych i bisfenolu A w eluatach ocenianych materiałów, niezależnie od czasu obserwacji. Uzyskane wyniki wskazują, że polimeryzowane światłem widzialnym ortodontyczne systemy adhezyjne są niestabilne chemicznie i w ciągu długiego czasu emitują do środowiska zewnętrznego składniki biologicznie szkodliwe. Materiały stomatologiczne zawierające usieciowane metakrylany wymagają modyfikacji struktury lub eliminacji szkodliwych komponentów w celu zwiększenia bezpieczeństwa ich stosowania.

Słowa kluczowe: ortodontyczne systemy adhezyjne, uwalnianie monomerów, HPLC.

With the development of orthodontic treatment techniques associated with using fixed appliances, it

became necessary to attach their elements to the teeth, whose movement they were supposed to cause. As early as in the mid-50s of the twentieth century attempts were made to use epoxy resins for fastening brackets to labial surfaces of teeth. Towards the end of the 60s of the last century, the first adhesive resins bonding dental materials with hard tissues of teeth were introduced to the market, which were based on methacrylate polymers. Soon after that, they started to be used in orthodontics. Currently applied orthodontic adhesive systems structurally resemble complex materials used for enamel and dentin fillings in conservative dentistry. Most of the modern orthodontic adhesive systems contain an organic polymer matrix, inor-

¹⁾ Medical University of Warsaw, Department of Orthodontics, ul. Nowogrodzka 59, 02-006 Warszawa, Poland.

²⁾ Medical University of Warsaw, Department of Oral Surgery, ul. Nowogrodzka 59, 02-006 Warszawa, Poland.

³⁾ Private practice.

⁴⁾ Medical University of Warsaw, Department of Pediatric Dentistry, ul. Miodowa 18, 00-246 Warszawa, Poland.

⁵⁾ Medical University of Warsaw, Chair and Department of Drug Chemistry and Pharmaceutical Biotechnology, ul. Banacha 1, 02-097 Warszawa, Poland.

^{*} Author for correspondence; e-mail: konrad.malkiewicz@interia.pl

ganic fillers, bonding agents and systems responsible for initiating polymerization reactions [1].

Today's clinical practice involves primarily orthodontic adhesives cured with visible light [2]. Orthodontic adhesive systems are polymerized in a radical reaction mechanism, where double monomer bonds are reacted and methacrylate molecules join together. Unfortunately, not all of the monomers forming the orthodontic adhesive systems are combined, and some of the double bonds are not consumed. After polymerization, orthodontic adhesive material contains free monomers, while certain molecules associated with the polymer network have active, unbonded functional groups, causing chemical instability of the material [3, 4].

The skeleton of the polymer material is composed mainly of methacrylates, of which the main component is bisphenol A-glycidyl methacrylate (Bis-GMA). Apart from Bis-GMA, orthodontic adhesives can contain urethane dimethacrylate (UDMA), triethylene glycol dimethacrylate (TEGDMA) and other methacrylate derivatives. Numerous reports confirm that the above mentioned compounds have potent cytotoxic, carcinogenic and genotoxic properties [5–8].

Particular attention is paid to the problem of bisphenol A release from polymer-based medical products, which is used in production of Bis-GMA resin — one of the basic components of dental composite materials. Bisphenol A is an organic chemical compound from the phenol group and is widely used in synthesis of plastics. Polycarbonates, which are a potential source of exposure to BPA, are used for production of, among other things, food storage containers, beverage bottles, dental materials, plumbing installations, tableware items, electronic devices, car parts, protective pads and helmets, and many other objects of everyday use.

Available literature has numerous reports confirming a pathogenic effect of BPA on the processes of human body's immune response, the functions of the male and female reproductive system, the maturation of the nervous system, the insulin management and the cardiovascular system. Bisphenol A is also believed to play a part in induction of neoplasms, in development of obesity and diabetes, and in causing neurological disorders. The BPA action mechanism includes, *inter alia*, mimicking the function of estrogen hormones. In addition to parahormonal action [9, 10], the compound demonstrates cytotoxic, mutagenic and carcinogenic properties [11, 12].

The extensive use of bisphenol A in production processes, and a wide spectrum of BPA action on living organisms, which has been confirmed by many studies, resulted in legislative changes both in European countries and the USA. Subsequent to action taken by governments of Denmark and France, and to introduction of new legislation by the European Commission, it is prohibited in the member states of the European Union to use products containing BPA for storing food and feeding children. The US Food and Drug Administration declares an im-

plementation of measures designed to remove bisphenol A from manufacturing processes of food packaging, as well as tightening of rules for its use.

Potential health risks associated with release of biologically toxic chemical compounds from medical products should trigger further studies assessing the safety of their use in the treatment process.

The aim of the study was an assessment of chemical stability of orthodontic adhesive systems in *in vitro* conditions.

EXPERIMENTAL PART

Materials

Four orthodontic adhesives commonly used in Poland were evaluated: ConTec LC (Dentaurum, Germany) Resilience (Ortho Technology, USA), Light Bond (Reliance Orthodontic Products, USA) and Transbond XT (3M, USA). The resins came from Polish distribution sources, and their sale on the Polish market had been authorized.

Preparation of samples

The studied orthodontic adhesives were placed in wells of Teflon matrices 5 mm in diameter and 2 mm deep. After filling the matrices, each sample was polymerized with the use of LED 55 lamp (TPC Advanced Technology, USA) for 40 seconds. The method described above was used to prepare 20 samples of each of the evaluated materials, which were then randomly divided into four groups (5 samples each), corresponding to different time periods of the planned experiment.

24 hours after polymerization the samples of studied materials were placed in glass vials filled with 10 cm³ of water (HPLC grade) with added 0.05 cm³ of Antibiotic Antimicrobial preparation (Invitrogen, USA). Five samples of each tested material were incubated for, respectively, 1 hour, 24 hours, 7 days and 30 days, and the resulting aqueous eluates were frozen at minus 8 °C.

Methods of testing

Chromatographic analysis

The samples were thawed immediately before chromatographic analysis by HPLC in a water bath at 37 °C.

The following chemical compounds were identified in the resulting aqueous solutions: HEMA/2-hydroxyethylmethacrylate, TEGDMA/triethylene glycol dimethacrylate, EGDMA/ethylene glycol dimethacrylate, UDMA/urethane dimethacrylate and BPA/bisphenol A, which were detected with the use of reversed phase high pressure liquid chromatography (HPLC). The concentration gradient was prepared on the basis of the method proposed by Manojlovic *et al.* [13] with own modification consisting in a change in the concentration gradient of water

and acetonitrile in the chromatographic system in order to separate the phases correctly. Identification of concentrations of chemical compounds eluted from composite materials stored in water was carried out by qualitative and quantitative analysis of the studied solutions. The resulting values were expressed in $\mu\text{g}/\text{cm}^3$.

Statistical analysis

The results were subjected to statistical analysis at the assumed significance level of $p = 0.05$.

For continuous variables the following values were calculated: number, arithmetic mean, standard deviation, median, minimum value and maximum value.

The basic tool in the analysis of the averages was the one-way analysis of variance (ANOVA one-way). Normal distribution was assumed. The assumption of equality of variance was tested with Brown–Forsthe test. Tukey test was used for multiple comparison.

RESULTS AND DISCUSSION

The average concentration of the most released components from the ConTec LC material

In ConTec LC material eluates obtained after one-hour incubation in water, TEGDMA was the dominant chemi-

Table 1. Mean concentrations of chemical compounds released from the ConTec LC material in respective time of observation

Identified substance	Concentration $\mu\text{g}/\text{cm}^3$	SD	Time of observation
HEMA	0.000	0.000	1 hour
EGDMA	0.001	0.003	
UDMA	0.035	0.022	
BPA	0.131	0.108	
TEGDMA	4.539	2.685	
EGDMA	0.036	0.036	24 hours
UDMA	0.040	0.039	
HEMA	0.090	0.091	
BPA	0.183	0.098	
TEGDMA	5.676	0.499	
UDMA	0.002	0.005	7 days
HEMA	0.106	0.048	
BPA	0.264	0.143	
EGDMA	0.714	0.306	
TEGDMA	5.390	1.567	
UDMA	0.000	0.000	30 days
BPA	0.046	0.024	
HEMA	0.593	0.416	
EGDMA	0.947	0.436	
TEGDMA	5.938	1.600	

SD – standard deviation.

cal compound released into the external environment at a statistically significant level ($p = 0.05$), and its concentration in the solution averaged $4.539 \mu\text{g}/\text{cm}^3$. After 24 hours the average concentration of TEGDMA was $5.676 \mu\text{g}/\text{cm}^3$ and was statistically significantly higher ($p = 0.05$) than concentrations of the other chemical compounds, ranging from $0.036 \mu\text{g}/\text{cm}^3$ for EDGMA to $0.183 \mu\text{g}/\text{cm}^3$ for BPA. In ConTec LC eluates obtained after 7 days of storage in water, the concentration of TEGDMA equaled $5.390 \mu\text{g}/\text{cm}^3$ and still remained significantly higher ($p = 0.05$) than those of the other identified substances. After 30 days of ConTec LC incubation in aqueous environment, TEGDMA remained the compound released in the highest statistically significant ($p = 0.05$) quantities, and its concentration in the solution was $5.938 \mu\text{g}/\text{cm}^3$, whereas concentrations of the other identified substances ranged from $0.000 \mu\text{g}/\text{cm}^3$ for UDMA, to $0.947 \mu\text{g}/\text{cm}^3$ for EGDMA.

The concentrations of chemical compounds released from the ConTec LC material in respective observation periods are shown in Table 1.

The average concentration of the most released components from the Resilience material

In the case of solution samples collected after one-hour storage of the Resilience material, the compound released in statistically significantly ($p = 0.05$) the greatest amounts compared to the other identifiable substances was TEGDMA, and its concentration was determined at the level of $1.939 \mu\text{g}/\text{cm}^3$. The concentrations of the other identified compounds ranged from $0.004 \mu\text{g}/\text{cm}^3$ for HEMA to $0.096 \mu\text{g}/\text{cm}^3$ for BPA. After 24 hours of Resilience sample storage in water, TEGDMA was still the chemical compound which was released into the external environment in statistically significantly ($p = 0.05$) the largest quantities, and its mean concentration amounted to $2.178 \mu\text{g}/\text{cm}^3$. The concentration of BPA in the solution obtained from the Resilience material was statistically significant ($p = 0.05$) at $0.400 \mu\text{g}/\text{cm}^3$, which was lower than the concentration of TEGDMA, but at the same time significantly higher ($p = 0.05$) than concentrations of the other chemical compounds. As far as the eluates obtained after seven days of storage in water of the assessed orthodontic adhesive, statistically significantly ($p = 0.05$) the highest concentration among the identified chemical compounds was observed in the case of the TEGDMA monomer at $2.731 \mu\text{g}/\text{cm}^3$. The concentrations of the other chemicals ranged from $0.003 \mu\text{g}/\text{cm}^3$ for UDMA to $0.414 \mu\text{g}/\text{cm}^3$ for BPA. After 30 days of observation TEGDMA concentration was $1.728 \mu\text{g}/\text{cm}^3$ and did not differ statistically significantly ($p = 0.05$) from EGDMA concentration at $1.695 \mu\text{g}/\text{cm}^3$. The concentrations of the other chemicals ranged from $0.004 \mu\text{g}/\text{cm}^3$ for UDMA to $0.487 \mu\text{g}/\text{cm}^3$ for HEMA and were statistically significantly lower ($p = 0.05$) than those of TEGDMA and EGDMA.

The concentrations of chemical compounds released from the Resilience material in respective observation periods are shown in Table 2.

Table 2. Mean concentrations of chemical compounds released from the Resilience material in respective time of observation

Identified substance	Concentration $\mu\text{g}/\text{cm}^3$	SD	Time of observation
HEMA	0.004	0.009	1 hour
EGDMA	0.020	0.002	
UDMA	0.035	0.022	
BPA	0.096	0.049	
TEGDMA	1.939	0.251	
HEMA	0.004	0.003	24 hours
UDMA	0.016	0.003	
EGDMA	0.025	0.003	
BPA	0.400	0.098	
TEGDMA	2.178	0.313	
UDMA	0.003	0.004	7 days
HEMA	0.157	0.061	
BPA	0.414	0.056	
EGDMA	1.166	0.272	
TEGDMA	2.731	1.113	
UDMA	0.004	0.010	30 days
BPA	0.038	0.020	
HEMA	0.487	0.102	
EGDMA	1.695	0.248	
TEGDMA	1.728	0.971	

SD — standard deviation.

The average concentration of the most released components from the Light Bond material

After one-hour storage of the Light Bond orthodontic adhesive resin in water, chemical compounds that were released in amounts statistically significantly higher ($p = 0.05$) than the others were UDMA ($0.694 \mu\text{g}/\text{cm}^3$) and TEGDMA ($2.868 \mu\text{g}/\text{cm}^3$). As far as solutions obtained after 24-hour incubation of the Light Bond material in water, UDMA (mean concentration of $2.225 \mu\text{g}/\text{cm}^3$) and TEGDMA (average concentration $2.779 \mu\text{g}/\text{cm}^3$) remained the substances whose concentrations were statistically significantly higher ($p = 0.05$) compared to the other identified chemical compounds. At the same time the UDMA concentration was statistically significantly lower ($p = 0.05$) than the concentration of TEGDMA. The eluates obtained after seven days of the Light Bond material storage in water again demonstrated the highest statistically significant ($p = 0.05$) concentrations of UDMA and TEGDMA (average concentrations of, respectively, $2.368 \mu\text{g}/\text{cm}^3$, and $2.772 \mu\text{g}/\text{cm}^3$) in comparison with the other identified substances. The concentration of the two compounds was comparable ($p = 0.05$) in this period of

observation. An analysis of the solutions performed after 30 days of incubation of the Light Bond orthodontic adhesive resin in an aqueous environment showed that the chemical compound present in statistically significantly ($p = 0.05$) the highest concentration compared with the other substances was TEGDMA with an average concentration of $2.113 \mu\text{g}/\text{cm}^3$.

The concentrations of chemical compounds released from the Light Bond material in respective observation periods are shown in Table 3.

Table 3. Mean concentrations of chemical compounds released from the Light Bond material in respective time of observation

Identified substance	Concentration $\mu\text{g}/\text{cm}^3$	SD	Time of observation
EGDMA	0.020	0.001	1 hour
BPA	0.024	0.013	
HEMA	0.083	0.072	
UDMA	0.694	0.244	
TEGDMA	2.868	0.202	
EGDMA	0.019	0.005	24 hours
HEMA	0.042	0.014	
BPA	0.133	0.042	
UDMA	2.225	0.135	
TEGDMA	2.779	0.411	
BPA	0.073	0.048	7 days
HEMA	0.133	0.044	
EGDMA	0.279	0.087	
UDMA	2.368	0.524	
TEGDMA	2.772	0.658	
BPA	0.009	0.008	30 days
EGDMA	0.288	0.046	
HEMA	0.467	0.132	
UDMA	0.926	0.300	
TEGDMA	2.113	0.209	

SD — standard deviation.

The average concentration of the most released components from the Transbond XT material

The chemical compounds present at statistically significantly the highest ($p = 0.05$) levels compared to the other substances observed in the solution after one-hour release from the Transbond XT material were UDMA (average concentration of $0.045 \mu\text{g}/\text{cm}^3$) and BPA (average concentration $0.052 \mu\text{g}/\text{cm}^3$).

After 24 hours of incubation of the orthodontic adhesive material in water, the substance which was released statistically significantly ($p = 0.05$) at the highest level proved to be BPA and its concentration in the solution was $0.255 \mu\text{g}/\text{cm}^3$. The eluates obtained from the Transbond XT material after seven days of observation demonstrated statistically significantly ($p = 0.05$) the highest

concentration for EGDMA (mean concentration of $0.906 \mu\text{g}/\text{cm}^3$) compared with the other identified chemical compounds. The substance emitted to the external environment at levels statistically significantly ($p = 0.05$) lower than EGDMA, but also statistically significantly ($p = 0.05$) higher than in the case of the other chemical compounds was BPA with the average concentration at $0.504 \mu\text{g}/\text{cm}^3$. After 30 days of storage of the Transbond XT samples in water, the average EGDMA monomer concentration equaled $1.321 \mu\text{g}/\text{cm}^3$ and was statistically significantly ($p = 0.05$) higher than the concentrations of the other identified compounds.

The concentrations of chemical compounds released from the Transbond XT material in respective observation periods are shown in Table 4.

Table 4. Mean concentrations of chemical compounds released from the Transbond XT material in respective time of observation

Identified substance	Concentration $\mu\text{g}/\text{cm}^3$	SD	Time of observation
EGDMA	0.000	0.000	1 hour
HEMA	0.000	0.000	
TEGDMA	0.013	0.030	
UDMA	0.045	0.008	
BPA	0.052	0.026	
EGDMA	0.000	0.000	24 hours
HEMA	0.002	0.002	
TEGDMA	0.005	0.003	
UDMA	0.025	0.012	
BPA	0.255	0.100	
TEGDMA	0.000	0.000	7 days
UDMA	0.001	0.004	
HEMA	0.080	0.017	
BPA	0.504	0.144	
EGDMA	0.906	0.147	
TEGDMA	0.000	0.000	30 days
UDMA	0.002	0.004	
BPA	0.157	0.064	
HEMA	0.369	0.078	
EGDMA	1.321	0.260	

SD – standard deviation.

Discussion

The results of the study confirm the thesis about chemical instability of orthodontic adhesive systems commonly used in clinical practice. Chromatographic analysis of eluates of the assessed dental materials showed that in laboratory conditions the polymerized orthodontic adhesives emitted a wide spectrum of biologically harmful chemicals to the external environment for the duration of the experiment. Available literature offers few reports describing the release of components from orthodontic

adhesive resins into the external environment. It is generally accepted that orthodontic adhesives based on methacrylate satisfy the safety conditions for their use, both in the case of treated patients and of medical staff administering them.

In 1995 Eliades *et al.* [14] reported release of TEGDMA and Bis-GMA from orthodontic adhesives into 75 % ethanol solution. The authors applied 100 mg of the evaluated adhesive resins, *i.e.* the light cured Transbond material (3M, USA), and the Concise adhesive (3M, USA) which was polymerized through a chemical reaction, on bases of steel and ceramic brackets. The test samples were eluted in 75 % ethanol for 15 days. The authors evaluated the presence of Bis-GMA and TEGDMA in solutions using high performance liquid chromatography. Eliades *et al.* [14] reported the presence of TEGDMA in the studied eluates at concentrations $2.6\text{--}174 \text{ mg}/0.01 \text{ cm}^3$, and of Bis-GMA at concentrations from 5.3 to $61.6 \text{ mg}/0.01 \text{ cm}^3$. The results obtained by Eliades *et al.* [14] confirm chemical instability of orthodontic adhesives, however, the concentrations of the identified chemical compounds described by the quoted authors raise certain doubts. It seems that the amount of the substance expressed in milligrams cannot fit in a volume of 0.01 cm^3 . Perhaps the authors included incorrect measurement units in the text.

Ten years later, a team led by Gioka [15] published a paper describing release of TEGDMA into the aqueous environment, where the chemical compound was derived from two different orthodontic adhesives: chemically cured Rely-a-Bond resin (Reliance, USA) and a light-cured resin from the same company (Reliance, USA), whose name was not provided by the authors. Samples of those materials were deposited on the bases of metal brackets and polymerized in accordance with the manufacturers' recommendations then placed for 2 months in 0.9 % saline. The eluates from the brackets and from the materials were analyzed by high performance liquid chromatography. For both chemo- and light-cured resin, Gioka *et al.* [15] confirmed the presence of TEGDMA in the solution in respective concentrations of 13.2 ppm and 11.5 ppm.

In the current study the presence of TEGDMA was identified in concentrations ranging from $2.113 \mu\text{g}/\text{cm}^3$ to $2,868 \mu\text{g}/\text{cm}^3$, which was released from the Light-Light Bond adhesive resin made by the same manufacturer, whose products were evaluated by Gioka *et al.* Possibly the quoted authors [15] also evaluated the Light Bond material or its previous generation. Unfortunately, the missing name of the orthodontic adhesive in the paper, a different method of sample storage and of analysis, as well as a significantly different observation time make a quantitative comparison of the results of both studies impossible.

In 2007 the team of Eliades *et al.* [16] published an article in which the authors evaluated the release of bisphenol A from orthodontic adhesives applied on steel bracket bases. Eliades *et al.* [16] used HPLC to assess BPA

emission to 99 % ethanol from chemically cured Rely-a-Bond adhesive (Reliance, USA) and a light-cured material made by the same company. It should be noted that the quoted authors, as was the case in the previously described publication, did not mention the name of the light-polymerized material whose properties they evaluated. We can only assume that — as in the previous study — it was Light Bond or the previous generation of the Reliance material. The study by the quoted authors lasted for 35 days. The eluates for chromatographic analysis were collected after one day, seven days, 21 days and 35 days of sample incubation in 15 cm³ of 99 % ethanol. Eliades *et al.* [16] ruled out the presence of bisphenol A in the studied samples above the level of 0.1 ppm, proposing the thesis that orthodontic adhesive resins do not emit potentially hazardous bisphenol A into the external environment. The results of the present study do not confirm the conclusion of Eliades *et al.* [16]. The chromatographic analysis of aqueous eluates performed in the present study showed a release of bisphenol A from samples of all evaluated orthodontic adhesives, including the Light Bond resin (Reliance, USA).

The results of the present study also contradict the argument about chemical stability of orthodontic adhesive resins, documenting a wide range of components released into the external environment from the assessed dental materials.

In an article published in 2011 by the team of Eliades *et al.* [17], the authors reported release of bisphenol A to the external environment from the Transbond XT adhesive (3M, USA) which was applied on bracket bases stuck to extracted premolars. Using the method of liquid chromatography and mass spectrometry, the authors identified BPA in the water in which the test materials were stored for 10, 20 or 30 days. Eliades *et al.* [17] confirmed the presence of BPA in the studied solutions at the level of no more than 2.9 µg/dm³, which is 0.0029 µg/cm³.

Our study also evaluated the emission of bisphenol A from the Transbond XT orthodontic adhesive resin into aqueous solutions. It confirmed presence of BPA in eluates from all periods of observation, and concentrations of the identified chemical compound ranged from 0.052 µg/cm³ to 0.504 µg/cm³, which was many times higher than the values reported by Eliades *et al.* [17] in 2011. Although it is impossible to directly compare the results of both studies in the quantitative context due to different methodologies used for assessing orthodontic adhesives, both studies confirm release of bisphenol A from the Transbond XT adhesive to the external environment. In the quoted publication the team led by Eliades [17] also points out that BPA in identified concentrations may be active in *in vivo* conditions. The above declaration constitutes a change in the authors' position as for biosafety of orthodontic adhesives which they demonstrated in their earlier publications.

The results of the study and a few publications by other authors suggest that orthodontic adhesive systems

are not chemically stable and release to the external environment substances which are potentially harmful to the health of patients and medical staff.

It is also alarming that manufacturers fail to provide full information about the composition of dental materials which may contain potentially harmful substances. It is therefore necessary to introduce legislation imposing on manufacturers an obligation to conduct thorough testing of medical materials before launching them into the market, impose standardization of methods for assessing the safety of their use in patients and publication of accurate and complete product documentation.

CONCLUSIONS

— In the conditions of the present study orthodontic adhesive systems polymerized with visible light remain chemically unstable.

— The release of biologically harmful components from orthodontic adhesives into the external environment is a long term process which may have a harmful effect on the health of patients.

— Polymer systems based on methacrylates, used for production of dental materials, require modification of structure, which would increase their chemical stability, or replacement by compounds with greater biosafety than in the case of the currently used materials.

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