International Journal of Occupational Medicine and Environmental Health, Vol. 11, No 4, 349 – 358, 1998

OCCUPATIONAL ALLERGY TO ALDEHYDES IN HEALTH CARE WORKERS. CLINICAL OBSERVATIONS. EXPERIMENTS

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Key words: Allergy, Contact dermatitis, Health care workers, Formaldehyde, Glutaraldehyde, Glyoxal, Tests on laboratory animals, Cytotoxicity

Abstract. The incidence of allergy to aldehydes (formaldehyde, glutaraldehyde, glyoxal) was examined in 280 health care workers suffering from skin lesions. Allergy was diagnosed in 64 (22.8%) patients. The majority of them (85.9%) were sensitive only to 1 aldehyde. Formaldehyde caused allergy slightly more frequently (13.9%) than glutaraldehyde (12.4%). Only 5 (1.9%) patients were sensitive to glyoxal. The irritant effect of aldehydes to the rabbit eye and skin was tested by the Draize and OECD methods. Formaldehyde and glutaraldehyde showed stronger irritant effect than glyoxal. The sensitizing activity of aldehydes was also confirmed in guinea pigs (using the Maximization Test and the OECD methods). Formaldehyde showed the strongest and most persistent reactions. Significantly higher eosinophil and basophil counts were found in the blood samples of the sensitized guinea pigs. Cytotoxicity of glutaraldehyde and glyoxal was tested on mouse 3T3-L1 fibroblasts by the Neutral Red Uptake and MTT Reduction Assay. It was shown that both aldehydes were cytotoxic, and that the cytotoxic effect of glutaraldehyde was stronger than that of glyoxal.

INTRODUCTION

Disinfecting formulations are mixtures of numerous chemicals which, at higher concentrations, irritate conjunctiva, respiratory mucosa and skin (15). Many disinfecting formulations comprise aldehydes: formaldehyde (HCHO), glyoxal (OHC-CHO) and glutaraldehyde (OHC-$(CH_2)_3$-CHO). Irritating and sensitizing properties of formaldehyde have already been known for a long time (7,12). Information on allergy to glyoxal are rather scanty (5,8). On the other hand, many authors point to increasing incidence of allergy to glutaraldehyde.

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Exposure to glutaraldehyde may result in contact dermatitis and asthma (3,4,9,20). Over the last 2 years, there has been a two-fold increase in the number of health care workers with suspected occupational dermatitis referred to The Nofer Institute of Occupational Medicine for medical consultation. Most of them reported the skin lesions following the contact with disinfecting formulations. Thus, we have attempted to assess the type and incidence of allergy to aldehydes in health care workers and to experimentally assay the sensitizing and irritant potential of the aldehydes.

MATERIALS AND METHODS

Patients

From January 1995 to December 1997, 280 health care workers who had been suspected of occupational contact dermatitis and referred to The Nofer Institute of Occupational Medicine for medical consultation, were subjected to dermatological examinations. All of them were patch-tested according to standard procedures (21) using the European standard, including 1% (aq.) formaldehyde, and the supplementary (Chemotechnique Diagnostic, Malmö, Sweden) allergen sets. In addition, they were tested with 0.1% thimerosal, 0.2% glutaraldehyde (pet) and 1% glyoxal (aq).

Reactions were read after 48 and 72 h using the following score for the results: '+' – diffuse confluent erythema with palpable infiltration, '+ +' – erythema and infiltration with papules and vesicles, and '+ + +' – strong erythema with papules--vesicles.

Irritant and sensitizing activity in laboratory animals

The irritant effect of formaldehyde, glutaraldehyde and glyoxal on the chinchilla rabbit eye and skin was tested by the Draize (6) and OECD (16,17) procedures. Tampons soaked with aqueous solutions of formaldehyde (30%, 20%, 10%, 2%, 1%), glutaraldehyde (10%, 5%, 2%, 1%) and glyoxal (30%, 20%, 10%) were fixed for 4 h to the skin under an occlusive covering at places with non-damaged and incised epidermis (from which hair had been previously removed). Water applied in the same manner at the other side of the animal was used as the control. The skin was inspected 1, 24, 48, 72 and 96 h after the termination of a 4-h exposure and the reaction was assessed using the Draize scale.

The eye irritability test comprised instilling aqueous aldehyde solutions (0.5% and 1% formaldehyde, 0.2% and 0.5% glutaraldehyde, and 0.5% and 1% glyoxal) into one eye of each rabbit. The instilled eye was compared with the other (control) eye of each animal after 1, 24, 72 h and on day 7.

The skin and eye irritation index, i.e. mean of the scores from all observations, was calculated for each concentration separately.

The sensitizing effect of aldehydes was tested on IMP D-H guinea pigs using the Magnusson-Kligman Maximization (11) and OECD (18) tests. The Magnusson-Kligman scale was used to assess the results. Basophil and eosinophil counts in the blood of the sensitized animals were compared with those in the controls.

Cytotoxic activity

Cytotoxicity of glutaraldehyde and glyoxal was tested on mouse 3T3-L1 fibroblasts using the following methods: Neutral Red Uptake (NRU) (cell membrane integrity as an endpoint) and 3-[5,3-Dimetylthiazol-2-yl]-2,5 diphenyltetrazolium bromide (MTT) assay (succinic dehydrogenase activity as an endpoint) after a 72-h incubation of the cells with different concentrations of the chemical tested (1,2,14,17).

RESULTS

Clinical examinations of the patients

The examined group of 280 health care workers comprised 255 women and 25 men aged from 21 to 64 years. There were 121 (43.2%) nurses, 52 (18.6%) physicians, 42 (15.0%) ward attendants, 36 (12.8%) laboratory technicians, 15 (5.4%) dental assistants, 7 (2.5%) dental technicians, and 7 (2.5%) other health care workers in that group.

Allergy to at least one of the aldehydes (formaldehyde, glutaraldehyde and glyoxal) was found in 64 (22.8%) workers, primarily women. Only 4 men (3 physicians and one ward attendant) were hypersensitive to aldehydes. The sensitization was most frequent among the nurses (28.9%), physicians (25.0%), and dental assistants 40.0%). None of the dental technicians was found to be hypersensitive to aldehydes (Table 1).

Table 1. Allergy to aldehydes among health care workers vs. occupation

Occupation	No.	%
Nurse (n = 121)	35	28.9
Physician (n = 52)	13	25.0
Ward attendant (n = 42)	3	7.1
Laboratory technician (n = 36)	7	19.4
Dental assistant (n = 15)	6	40.0
Dental technician (n = 7)		
Other (n = 7)		
Total (n = 280)	64	22.8

Table 2 gives positive results of the patch tests with aldehydes. Allergy to formaldehyde (13.9%) was more frequent than allergy to glutaraldehyde (12.4%). Only 5 (1.9%) patients were sensitive to glyoxal. The majority of tests with aldehydes were positive (+ +) (38.7%) or strongly positive (+ + +) (33.3%). Weakly positive (+) test results were most frequent for glutaraldehyde (Table 3).

Reaction to only one aldehyde was evidently more frequent than to 2 or 3 aldehydes. As much as 55 (85.9%) patients reacted to one aldehyde, 7 (10.9%) patients reacted to two compounds (6 to formaldehyde and glutaraldehyde, 1 to glutaraldehyde and glyoxal). Two patients were sensitive to all three aldehydes.

Table 2. Positive results of patch tests with aldehydes in health care workers vs. occupation

Occupation	Formaldehyde n (%)	Glutaraldehyde n (%)	Glyoxal n (%)	Total n
Nurse (n = 121)	21 (17.3)	20 (16.5)	4 (3.3)	45
Physician (n = 52)	6 (11.5)	7 (13.5)		13
Ward attendant (n = 42)	1 (2.4)	1 (2.4)	1 (2.4)	3
Laboratory technician (n = 36)	7 (19.4)			7
Dental assistant (n = 15)	2 (13.3)	5 (33.3)		7
Total (n = 266)	37 (13.9)	33 (12.4)	5 (1.9)	75

Table 3. Intensity of positive results for patch tests with aldehydes

Aldehyde	Weak positive (+) n (%)	Positive (++) n (%)	Strong positive (+++) n (%)	Total
Formaldehyde	5 (13.5)	19 (51.4)	13 (35.1)	37
Glutaraldehyde	14 (42.4)	9 (27.3)	10 (30.3)	33
Glyoxal	2 (40.0)	1 (20.0)	2 (40.0)	5
Total	21 (28.0)	29 (38.7)	25 (33.3)	75

In total, the health care workers reacted positively in the patch tests to 43 different chemicals. In addition to aldehydes, other most frequent sensitizers included metals (21.8% of patients were hypersensitive to nickel, 9.6% to thimerosal – an organic compound of mercury, 7.5% to cobalt, and 5.7% to chromium), fragrances (10.1%), and thiuram rubber accelerators (6.4%).

From the clinical examinations and patch test results, allergic contact dermatitis was diagnosed in 175 (62.5%), irritant contact dermatitis in 87 (31.1%), dermatitis atopica in 11 (3.9%), and urticaria chronica in 7 (2.5%) patients.

Results of the experiments

Irritant effect of the aldehydes (Table 4)

The 30% and 20% aqueous solutions of formaldehyde applied to rabbit skin resulted in erythema- and oedema-type reactions. The skin irritation index (SII) values were above 2 scores in the Draize scale (6). Weak skin reactions were found after application of 10% formaldehyde and also of 10% and 5% glutaraldehyde. Formaldehyde or glutaraldehyde concentration of 2%, causing weak, short-lasting

Table 4. Results of aldehyde irritant activity tests in rabbits. Skin irritation index (SII) and eye irritation index (EII)

Chemical	Skin				Eye			
	Concentration (%)	SII	Persistence of reaction (days)	Rating	Concentration (%)	EII (days)		Rating
						1–3	7	
Formaldehyde	30	2.6	8	moderate	1.0	2.5	1.1	weak
	20	2.4	8	moderate				
	10	1.5	5	weak	0.5	0.5	0	
	2	0.5	2	weak				
	1	0	0	0				
Glutaraldehyde	10	1.5	6	weak	0.5	10.0	5.8	weak
	5	1.3	4	weak				
	2	0.5	2	weak	0.2	0.5	0	
	1	0	0	0				
Glyoxal	30	1.0	3	weak	1.0	0.2	0	< weak
	20	0.5	1	weak	0.5	0	0	
	10	0	0	0				

inflammatory reactions, was assumed to be the threshold value. Glyoxal was a weaker irritant. Its threshold value was 20%. Aldehydes, when applied at higher concentrations, caused skin discolouration. Formaldehyde stained the skin to violet grey, glutaraldehyde to orange, and glyoxal to yellow colour. Formaldehyde and glyoxal (1%) and glutaraldehyde (0.5%) dropped into the eye caused temporary conjunctivitis.

Sensitizing activity of the aldehydes (Table 5)

It was found that 1% formaldehyde and glyoxal, and 0.4% to 0.5% glutaraldehyde were most suitable for testing allergy to those compounds. Sensitization of the animals with the chemicals at these concentrations caused that 70% to 90% of them reacted in the challenge patch test. It was not possible to sensitize guinea pigs using glutaraldehyde or glyoxal at 1/2 of the concentration values specified above (0.2% and 0.5%, respectively).

Table 5. Results of aldehyde sensitizing activity tests in guinea pigs

Chemical	Concentration (%)	Sensitized animal (%)	Rating	Intensity allergic reaction	Persistence of reaction (days)
Formaldehyde	1	81	extreme	3–4	4
	0.5	33	moderate	1–2	3
	0.1	50	moderate	1	1
Glutaraldehyde	0.5	50	moderate	1–2	3
	0.4	71	strong	1	3
	0.2	0	0	0	0
Glyoxal	1.0	90	extreme	0.5–3	3
	0.5	0		0	0

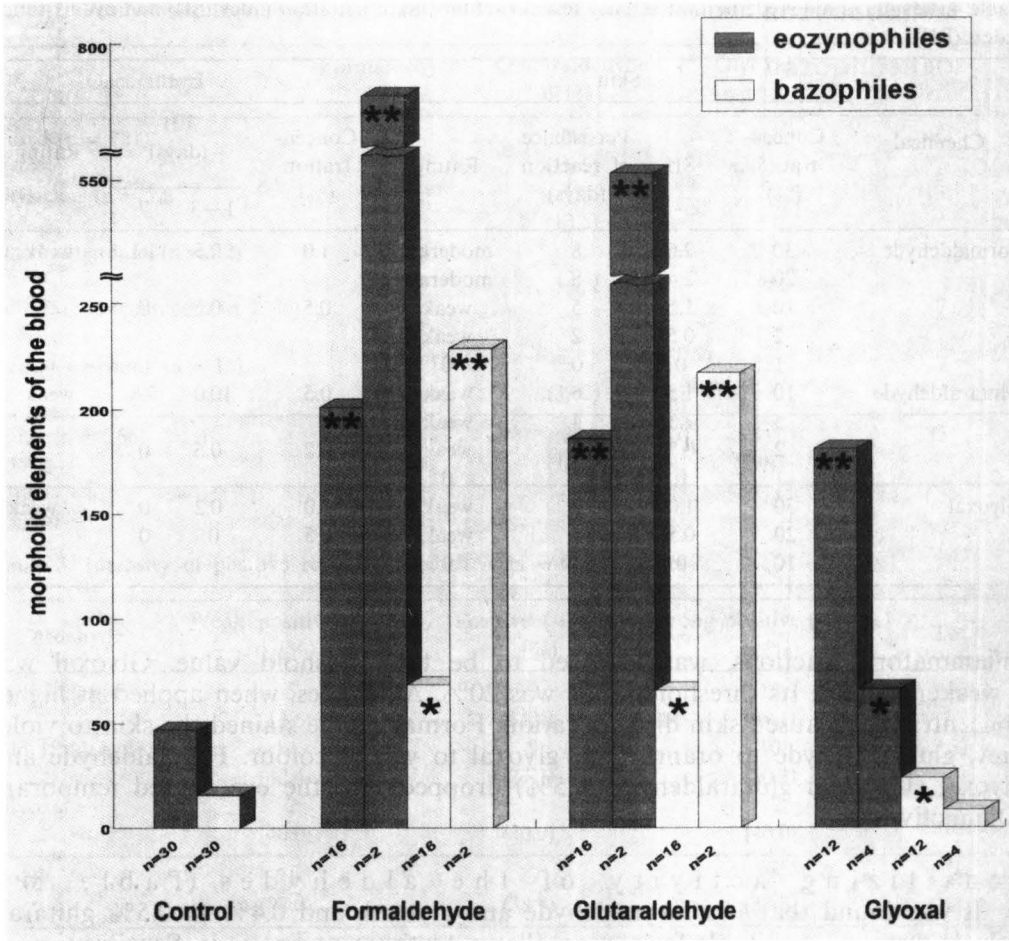

Fig. 1. Number of eozynophiles and bazophiles in 1 mm^3, peripheral blood of the guinea pigs; sensitized aldehydes (24 h after challenge).

* significantly different from the control $p < 0.05$

** significantly different from the control $p < 0.01$

Statistically significant increase in the number of eosinophils and basophils were observed in the sensitized animals (Fig. 1).

Cytotoxicity

Fig. 2 shows the results of cytotoxicity evaluation of glutaraldehyde using the NRU and MTT assays. Cytotoxicity of this aldehyde, assessed by means of the NRU assay, expressed as the effective concentrations causing 50% of the cell membrane damage (EC_{50}), was 0.0185 ± 0.0046 μg/ml. Cytotoxicity, expressed as the effective concentration causing reduction in succinic dehydrogenase activity by 50% was similar (0.019 ± 0.006 μg/ml).

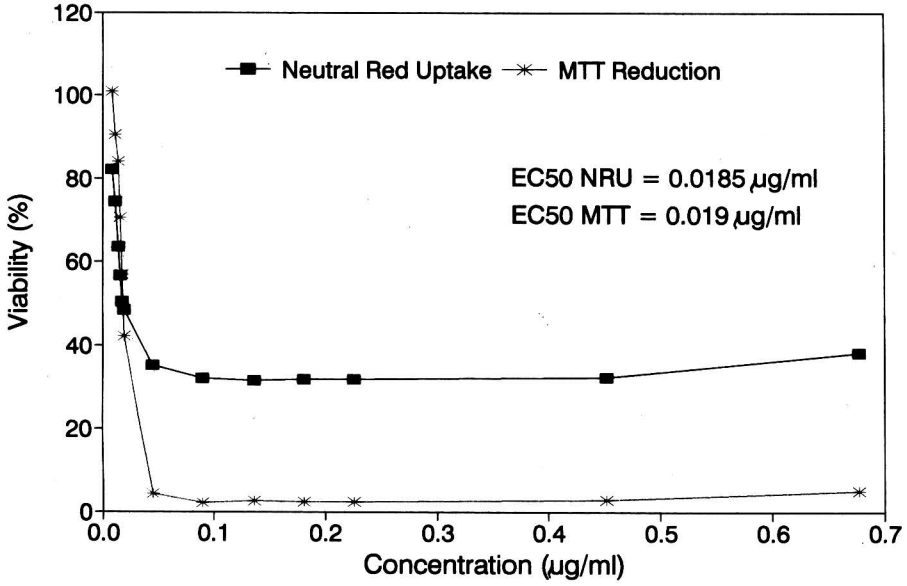

Fig. 2. Cytotoxicity of glutaraldehyde for 3T3-L1 mouse fibroblasts evaluated using Neutral Red Uptake and MTT Reduction Assays. Low cytotoxicity in NRU above concentration of 0.05 µg/ml seems to be caused by a cross-linking effect of aldehydes on the cell membrane protein, and is false negative.

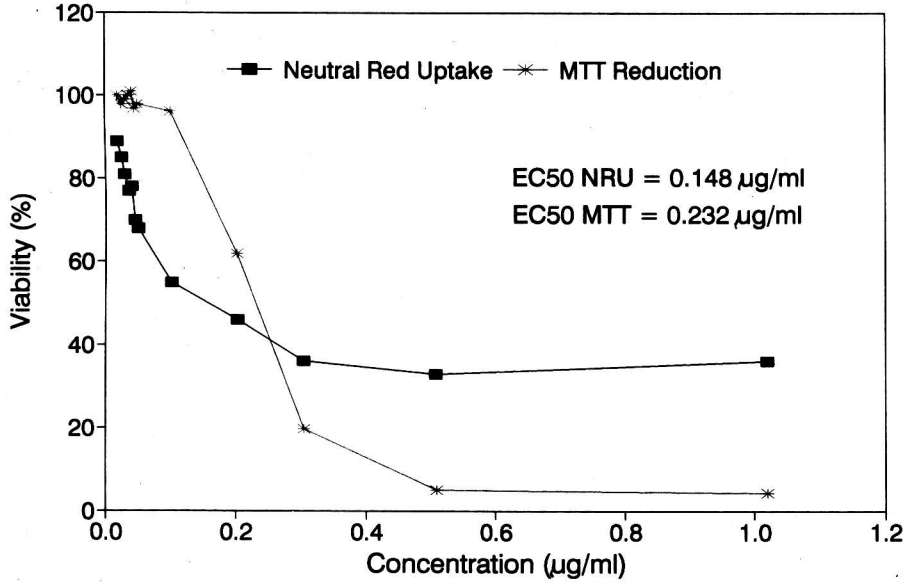

Fig. 3. Cytotoxicity of glyoxal for 3T3-L1 mouse fibroblasts evaluated using Neutral Red Uptake and MTT Reduction Assays. Low cytotoxicity in NRU above concentrations of 0.3 µg/ml seems to be caused by a cross-linking effect of aldehyde on the cell membrane protein, and is false negative.

Fig. 3 presents the results of cytotoxicity evaluation of glyoxal using the NRU and MTT assays. EC_{50} concentrations for this aldehyde were 0.148 ± 0.029 μg/ml and 0.232 ± 0.071 μg/ml, respectively. The results show that glutaraldehyde is by one order of magnitude more cytotoxic than glyoxal.

DISCUSSION

Aldehyde-based disinfecting formulations are widely used due to their excellent bactericidal, viricidal and fungicidal properties of disinfecting tools and surfaces. As much as 36 aldehyde-based disinfectants made in Denmark, England, Germany and Poland are licensed for use in Poland. Of these, 32 contain glutaraldehyde, 16 glyoxal, 5 formaldehyde; some of the formulations contain 2 or even 3 aldehydes.

In recent years there has been a continuing increase in the number of occupational skin diseases diagnosed in Polish health care workers. The increase is thought to be primarily due to widespread use of disinfectants, especially those containing aldehydes. Aldehydes are irritant to the conjunctiva, mucosa and skin. Their toxicity has been confirmed by the results of our *in vitro* experiments with mouse fibroblasts and *in vivo* tests on laboratory animals.

Aldehydes, especially formaldehyde and glutaraldehyde, are also known to produce allergic reactions (4,8,9,20). As much as 22.8% of our patients are hypersensitive to those chemicals. Although the use of formaldehyde in health care units has recently been reduced, the incidence of allergy to that chemical continues to be high. This observation is difficult to explain. Our results do not confirm cross-reactions to aldehydes, chemicals of similar chemical structure: 85.9% of our patients were sensitive to only one aldehyde. Therefore, it is reasonable to assume that sensitization to individual aldehydes develops separately for each chemical. It is quite probable that some formaldehyde-based disinfectants which continue to be marketed in Poland (A-10, Orochemie-Dürr + Pflug GmbH and Co KG, Germany; Desoform and Medisan 2000, Lysoform Dr Hans Rosemann GmbH, Germany; Prontocid, B.Braun Melsungen AG, Germany; Sekusept forte, Henkel Hygiene GmbH, Düsseldorf, Germany) have been frequently added to other disinfectants and resulted in a high incidence of allergy to formaldehyde. Also, it would not be reasonable to exclude a possible effect of non-occupational exposures. Formaldehyde is currently present in glues, plastics, paper, textile products, washing and cleaning agents (7).

The incidence of sensitization to glutaraldehyde tends to increase. Until 1993, only 1 case of allergy to glutaraldehyde was diagnosed, while recently we have diagnosed 11 cases of this allergy per year on average. Our results of glutaraldehyde cytotoxic, irritant and sensitizing activity tests, performed on the laboratory animals, and the increasing incidence among the medical personnel show that the problem is serious. Considering that the chemical is extensively used in health care units in Poland, it is reasonable to expect further increase in the incidence of hypersensitivity to glutaraldehyde, the more so that rubber gloves do not offer sufficient protection for the skin of the hands. Glutaraldehyde penetrates through rubber gloves (10). In addition to hospitals and consultation rooms, the agent is also used in scanning electron microscope (SEM) laboratories and by hairdressers to disinfect the instruments. It is also contained in photographic developers and leather tanning

formulations (12). Used in some topical medicaments, it may result in non-occupational allergy (13).

Reports on allergy to glyoxal are very rare (5,8). Glyoxal allergy usually accompanies hypersensitivity to formaldehyde or glutaraldehyde. In our study, five patients reacted to the chemical, of which only two reacted to glyoxal alone, while the remaining three were sensitive to glyoxal and other aldehydes. Experimental results confirm its weak irritant and sensitizing effect. Allergy to other chemicals found in the occupational environment, such as metals and rubber, is also frequent among Polish health care workers.

ACKNOWLEDGEMENTS

This work was performed under the Strategic Governmental Programme 'Safety and health protection in the work environment' co-sponsored by the State Committee for Scientific Research during the years 1998–2001. General co-ordinator: Central Institute for Labour Protection, Warsaw, Poland.

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Received for publication: October 21, 1998
Approved for publication: November 15, 1998

DIARY OF CONFERENCES AND SEMINARS - contd

6–8 October 1999, Vienna Austria
25th International Symposium on Safety and Health in the Construction Industry in the 21st Century
Info: Office for International Relations and Conferences Allgemeine Unfallversicherungsanstalt, Adalbert-Stiffer-Strasse 65, A-1200 Vienna, Austria
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The symposium is being organised by the International Section for the Prevention of Occupational Risks in the Construction Industry of the International Social Security Association (ISSA) and the Allgemeine Unfallversicherungsanstalt (AUVA).
Main topics:
- co-ordination of safety and health at work
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10–14 October 1999, Herzlia, Israel
14th International Conference on Epidemiology in Occupational Health
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Main topics:
- occupational work related diseases
- occupational cancer
- occupational epidemiology
- molecular epidemiology
- biological markers
- value of pre-employment examinations
- exposure assessment
- reproductive health studies
- communicable diseases.