Assessment and Measurement of the Occupational Exposure to Cytostatic Agents in Pharmacy Departments

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Introduction

The toxic side effects of cytostatic agents have been described extensively. Much attention has been paid to the carcinogenic, mutagenic and teratogenic properties of these compounds. For this reason, the preparation of the drugs is done inside so called work benches (Laminar air flow benches). These LAF benches should prevent personal, environment and the drug itself from getting contaminated or cross contaminated. In spite of careful handling inside those benches, contamination of the room and the personal could occur. This is shown by several measurements [1] and it is assumed that not only particles but also vapor of cytostatic compounds are emitted. Due to this assumptions we have measured the vapor pressure (vp) of some compounds (i.e. cyclophosphamide, fluorouracil). The vp of the investigated compounds were found to be in the range of some mPa. Based on this measurement calculation can be done to assess the evaporation time of particles. It was found that particles of cyclophosphamide, 50 µm in size, evaporate within nearly 1 day and the evaporation time of smaller particles is much more less. This is true even if the particles are captured in a high efficiency filter. Due to these considerations it is quite reasonable that there could be not only a contamination of the work environment by particles but also by vapor of cytostatic compounds.

Investigated Cytostatic Compounds

In a first step two of the mostly used substances in Germany i.e. fluorouracil {(5-Fluoro-2,4(1H,3H)pyrimidine-dione), C4H3FN2O2, mw 130,08} and cyclophosphamide {2-[bis(2-chloroethyl)-amino]-1,3,2-oxazaphosphorine- 2- oxide, C7H15Cl2N2O2P, mw 261,10} were investigated. The molecular structures are shown in figs. 1a, 1b.

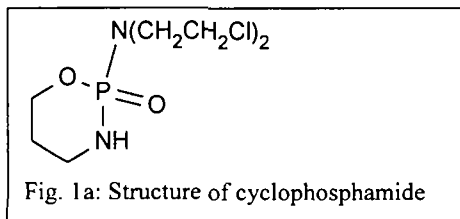

Fig. 1a: Structure of cyclophosphamide

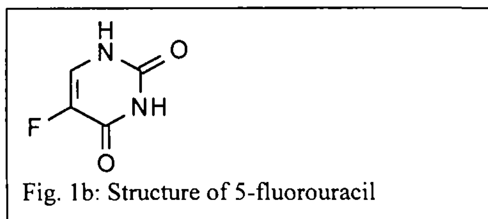

Fig. 1b: Structure of 5-fluorouracil

There are a lot of further cytostatic substances (more than one hundred). To get an image of some of those compounds the molecular structures of etoposide {4-o-Demethyl-1-1 o-(4,6-o-Ethylidene-ß-D-glucopyranosyl)-epi-podophyllotoxim, C29H32O13, mw 588,58} and methotrexate {4-Amino-4-desoxy-10-methyl-folic acid, C20H22N8O5, mw 454,46} are shown additionally in figs. 1c, 1e.

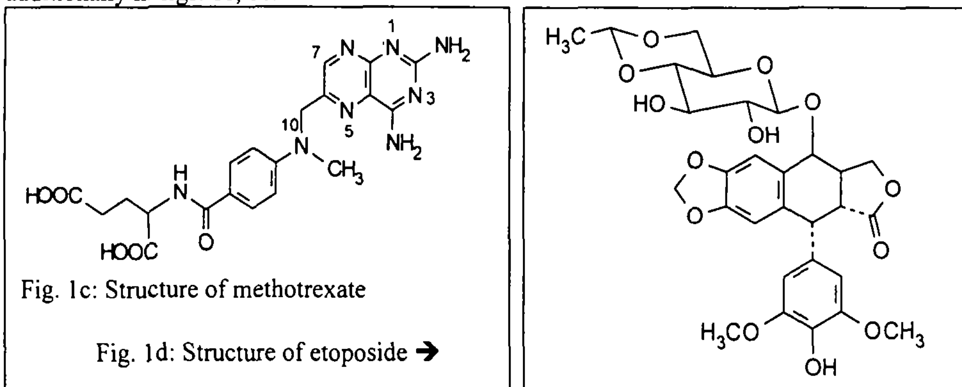

Fig. 1c: Structure of methotrexate

Fig. 1d: Structure of etoposide ➔

Measurement of the vp and assessment of the evaporation rate of particles consisting of 5FU and CP

The most important physical property concerning the evaporation rate is the vp. Unfortunately in the literature and in the data sheets of those cytostatic substances the vp is not mentioned. For this reason the vp's of the two substances 5FU and CP were measured using well known measuring procedures, i.e. effusion method, vapour pressure balance [2]. The measurements were done by HÜLS AG Marl, Germany. The vp was measured at different temperatures and the results of the measurements are shown in table 1 [3].

Table 1:

T / °C	Vapour pressure / mPa	
	CP	5 FU
20	3.3	1.4
40	9.0	3.9

Table 2:

Particle size / µm	Evaporation time / s	
	T=20°C	T=40°C
5	722	256
50	72200	25600

From the vp of substances the evaporation rate for particles of a certain size can be calculated [4]. To get an idea of the time scale particles need to evaporate the evaporation time of particles 5 µm and 50 µm in size was estimated. The result of the calculation for CP particles is shown in table 2. The most important result is that the evaporation process of a 50 µm particle at room temperature is terminated within a time scale of only one day and smaller particles evaporate much more faster. Thus, the evaporation of particles of cytostatic compounds comes down in the same time scale the drugs are prepared in the work benches. The question is how much particles are produced during preparation inside the work benches. Measurements concerning this problem will be done in the near future.

Assessment of the maximum vapour concentration of cytostatics in a room

From the vp, the molecular weight, the gas constant and the temperature the equilibrium vapour concentration in the ambient air of particles can be calculated [4]. Using the vp-values and the

molecular weights of the two investigated cytostatic compounds the equilibrium vapour concentrations at room temperature are found to be 360 µg/m³ and 80 µg/m³ for CP and 5FU respectively. Due to Dalton's law these concentrations have to be added to get the total vapour concentration of the cytostatic compounds, i.e. 440 µg/m³. This calculated concentration has to be regarded as the theoretical maximum concentration that is possible and such a value can only be reached in a closed and not ventilated room and without any losses e.c. by adsorption or reaction. Real measurement inside a workbench and in the ambient room (pharmacy department) will be done in the near future.

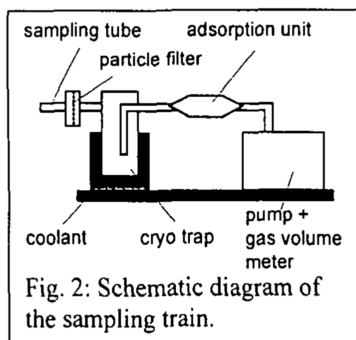

Fig. 2: Schematic diagram of the sampling train.

Up to now a method to take sample of the vapour and the particle phase of cytostatic compounds in rooms is prepared. The sampling train consists of a particle filter, a cryo trap and an adsorption unit for gaseous components (see fig. 2). In the laboratory we analyse the particle filter, the condensed liquids and solids of the cryo trap and the adsorption unit. For the quantitative measurement of the amount of cytostatic compounds we have prepared the chemical analysis methods to measure and quantify particle concentrations as well as vapour concentrations.

Analysis of cytostatic compounds

A) Chromatographic analysis of Cyclophosphamide.

Gas chromatographic methods for the determination of oxazaphosphorines have been published in a large way. Cyclophosphamide (CPA) is mostly derivated prior to gas chromatographic analysis by reaction with trifluoroacetic anhydride in main case for one reason: CPA decompose in the injector of the gas chromatograph, yielding two separate product peaks, one for CPA and another for an intramolecular cyclization product.

The first step of sample preparation includes the extraction of the sample with ethyl acetate (three times). The ether layers are combined, the internal standard is added followed by the evaporation under nitrogen at 70°C. Then 100 µl of ethyl acetate are used to solute the residue totally. Trifluoroacetic anhydride (100 µl) is added and - after mixing - the tubes were closed for derivation (1 hour, 70°C). The sample is cooled to room temperature and in a further step the solvent is caused to evaporate. The residue is dissolved in 100 µl toluene and filled in vials for analysis.

The sample prepared in the mentioned way is analysed on a Fisons GC-MS quadrupole system with selected-ion monitoring (SIM). Quantification of the N-trifluoroacetyl derivatives is performed on the base peak at m/z 307 in EI+-mode. Separation is carried out on a fused-silica capillary column (DB-5, 40 m * 0.25 mm I.D., film thickness 0.25 µm). The initial oven temperature is set to 70° C for 5 minutes and the temperature is increased by 10°C/min to 280°C, where it remains constant for another 10 min. Helium is used as carrier gas. Applying this method of analysis a detection limit of about 10 ng/l for CPA can be achieved.

B) Capillary electrophoretic analysis of fluorouracil and methotrexate.

Separation and determination of fluorouracil and methotrexate were performed by capillary electrophoresis. A CE Model P/ACE system 5000 Series 1 (Beckman Instruments, Fullerton,

CA, USA) with Diode Array Detector, detection between 190 and 350 nm, is used. All determinations are carried out in a 75 µm I.D. capillary with an effective separation length of 50 cm.
To prepare the capillary for measurement and to clean the surface between each analysis, the capillary was washed with 0,1 M NaOH, with 0,1 M HCl and with borate buffer (c = 0,01 mol/l, pH 9,39). The separation voltage was 25 kV and the current was about 30 µA while using borate buffer.
The detection limit using the DAD is in the range of 200 µg/l for methotrexate and fluorouracil. In the near future we will improve the detection limit by a factor of about 500 using a laser induced fluorescence spectrometer.

C) Liquid Chromatographic Analysis of further seven cytostatic compounds
A trace analytical procedure for the cytostatic drugs cyclophosphamide, methotrexate, 5-fluorouracil and carmustine, chlorambucil, cisplatin, cytarabine, etoposide, melphalan and vinblastine was developed using solid phase extraction with subsequent HPLC separation [5]. Identification and determination of the substances were performed by using DAD and fluorescence detection. For the enrichment procedure different solid phase materials i.e. C18 Polar Plus and SDB1 (Mallinckrodt Baker) were used. The HPLC-system consisted of two HPLC-pumps (LC-10AS), an UV diode array detector (SPD-M10A), a fluorescence detector (RF-10A) and a communication bus module (CBM-10A, all Shimadzu). The analytes were separated an a Nucleosil C-18-column (5 µm, 250x4.0 mm, Machery-Nagel) with a guard column of the same material (11x4mm). Separation was carried out using a binary gradient with a stepwise increasing percentage of methanol. For the aqueous part of the eluent the pH of a 0.01 M KH_2PO_4 buffer was adjusted to 3.0 with phosphoric acid. Gradient: 0-2 min: 3% methanol; 2-22 min: 43% methanol; 22-35 min: 75% methanol. Detection limits after the clean-up and enrichment procedure vary from 0.002 to 0.2 mg/l.

Results and Conclusion

Up to now we have done some preliminary measurements. We have taken samples in three different locations, one in a hospital pharmacy department and the others in two public pharmacy departments. At one workbench sample was taken directly in the air outlet of the bench. At the day of measurement Cytarabin was prepared mostly and we have found this substance in the adsorption unit of our sampling train (nothing was found neither in the cryo trap nor at the particle filter). The determination of this substance is given by a confidence of 80%.
The sampling at the other two benches was carried out quite close to the head of the pharmacist, which prepared the drugs. In one case the bench was equipped with a so called ventilation unit, that sucks away the outlet air of the bench. The result of the analysis of the sampling train is as follows: no substance was found neither at the particle filter, nor in the cryo trap nor in the adsorption unit.
In the other case the outlet air of the bench was re-circulated into the pharmacy room. The sampling was taken once more close to the pharmacists head. The result of the analysis is as follows: We have found at lot of different substances (20-30) at the particle filter, as well in the cryo trap as well in the adsorption unit. Some substances were found at the particle filter and not in the adsorption unit and vice versa. Unfortunately we were not able to identify each of the substances and we are not quite sure if cytostatic substances were included. Due to possible

unknown side effects the identification of the substances keeps very difficult. For that reason we will repeat some of the measurements under more defined conditions to deliver data of 100% confidence. Such data are needed to make clear decisions if something should be done in the field of environment and work protection concerning the preparation of cytostatic drugs.

Acknowledgement
The authors kindly acknowledge the support of the work by the state ministries of Nordrhein-Westfalen, i.e. MAGS, MWF, MURL, MWMTV. Further on the investigations were supported partly by different national foundations of Germany i.e. AIF, DBU.

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