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Cytotoxic drugs and the aquatic environment: estimation of bleomycin in river and water samples

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Abstract—A radioimmunoassay has been used to determine levels of the anticancer drug bleomycin in sewage treatment works effluent, river and potable water samples. Samples were concentrated 100-fold by lyophilisation and a final limit of detection of 5 ng L^{-1} was achieved. Concentrations of immunoreactive bleomycin of between 11 and 19 ng L^{-1} were found in the effluents but a lower concentration range < 5–17 ng L^{-1} was found in river and potable water samples. The risk to human health of ingesting water (in SE England) with such low levels of this cytotoxic drug appears to be minimal in relation to the normal chemotherapeutic doses administered (20–30 mg m^{-2}).

The risks associated with the disposal of pharmaceutical wastes have been discussed in detail (Richardson & Bowron 1985; Lee 1988). The disposal of prescription-only medicines, including cytotoxic drugs, should comply with the Control of Pollution (Special Waste) Regulations 1980 no matter how small the discharge. However, the Department of Environment Circular 4/81, indicates that a commonsense approach should be adopted and that householders can dispose of small quantities of unwanted pharmaceuticals by flushing to sewer. Waste Disposal Authorities are also advised to adopt a similar *de minimus* approach in the industrial and commercial sector where appropriate (DOE 1983; HSC 1982).

Cytotoxic drugs used for the treatment of cancer and immunosuppressive agents are perhaps some of the most toxic chemicals available to the public. They are administered mainly in hospital and by medical practitioners, but also to a lesser extent in the home. Whilst discharge to the aquatic environment can easily be controlled during manufacturing procedures, their disposal from the hospital, surgery or home is less easy, if not impossible, to control.

Many antineoplastic drugs have been reported to be carcinogenic, mutagenic and teratogenic. The precautions to be taken when handling injectable antineoplastic drugs have been discussed (Knowles & Virden 1980) and guidelines have been published by both the Royal Pharmaceutical Society of Great Britain (1983) and the Health and Safety Executive (1983). The potential hazards of occupational exposure to cytotoxic agents have been the cause of concern for some time and there may be a causal relationship between the handling of anticancer drugs and foetal abnormalities in pregnant nurses (Hemminki et al 1985; Selevan et al 1985). Detectable amounts of cyclophosphamide have been found in the urine of nurses handling the drug (Hirst et al 1984). Concern has also been expressed that the disposal of cytotoxic drugs could present a risk to potable water resources particularly those derived from rivers receiving discharges from sewage treatment works.

Very little work has been carried out to investigate this potential risk. Concentrations of methotrexate of approximately 1 μg L^{-1} have been found in a sewer immediately downstream of an oncology clinic but sewage and water treatment, dilution and degradation effects reduced this level in all river and potable water samples tested to less than 6·25 ng L^{-1} (Aherne et al 1985).

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Methotrexate is known to be readily metabolized and to undergo hydrolytic decomposition (Gallelli & Yokoyama 1967) and is therefore not an ideal model compound. In view of this, a further study has been carried out using the widely used anticancer drug bleomycin as a representative compound. Bleomycin was selected from drugs cited by Lee (1988) and from others listed in Martindale (1989) as it is relatively stable, used in moderately high doses (20–30 mg m^{-2} per dose) and can be specifically assayed at low concentrations using a readily available radioimmunoassay (Teale et al 1977).

Materials and methods

Snap water samples from both activated sludge and conventional filter sewage treatment works effluents, rivers and drinking water supplies were collected from areas of SE England and stored at 4°C in glass bottles. Samples were collected between February and March 1989. Amounts (25 mL) of each sample were lyophilized in glass containers and the residues stored at −20°C until assayed. For control samples, HPLC grade water (Aldrich Chemical Co) was spiked with bleomycin (Blenoxane, Lundbeck) at a concentration of 100 or 200 ng L^{-1} and freeze dried in a similar manner. The radioimmunoassay described by Teale et al (1977) was established using ^{125}I-labelled bleomycin (Aherne et al 1982), and modified so that 500 μL of each undiluted water sample could be assayed.

The residues from the lyophilized water samples were resuspended in 250 μL double-distilled water, the liquid with suspended solids transferred to small conical tubes and centrifuged for 2 min in a microfuge (Eppendorf) and 100 μL amounts of the supernatants were taken in duplicate for inclusion in the unmodified radioimmunoassay. This procedure represents a 100-fold concentration step for each sample.

Results

Using a modified assay in which the volume of standards and samples used was increased from 100 to 500 μL the limit of detection of the assay (determined by a 10% fall in binding from that of the zero standard binding, B_0) was 0·06 ng mL^{-1} (60 ng L^{-1}). The recovery of bleomycin was 85% at an added concentration of 200 ng L^{-1}. Using the modified assay, 7 sewage effuent samples showed positive immunoreactivity.

The results obtained on the reconstituted samples are shown in Table 1. The limit of detection of the standard curve was 5 ng L^{-1} (10% fall from B_0) and the recovery of bleomycin was 79% at a spiked concentration of 100 ng L^{-1}.

Discussion

A radioimmunoassay for bleomycin, primarily designed for human pharmacokinetic studies, has been adapted for screening water samples for the presence of bleomycin immunoreactivity. Results have been expressed in terms of immunoreactivity as it is possible that there are other compounds present in water samples which may interfere with the antigen : antibody reaction. Indeed the large amount of suspended solids present after lyophilization may also cause some interference in the assay.

Table 1. Radioimmunoassay of bleomycin in water samples following concentration by lyophilization.

	Immunoreactive bleomycin ng L^{-1}	
Samples taken from:	Range	Mean (s.d.)
Sewage treatment works effluents (n = 9)	11–19	15·8 (3·0)
Rivers (n = 11)	< 5–17	8·5 (3·7)
Potable water (n = 9)	5–13	8·7 (3·3)

Recovery of bleomycin 79%: limit of detection 5 ng L^{-1}.

Six of the sewage treatment works effluents showed immunoreactivity when 500 μL of samples were assayed and in general the effluents showed higher concentrations of bleomycin immunoreactivity (15·8 ng L^{-1}, s.d. = 3·0) than either the river (8·5 ng L^{-1}, s.d. = 3·7) or potable water (8·7 ng L^{-1}, s.d. = 3·3) samples. The concentrations of bleomycin measured in these samples are approximately 1000-fold less than the plasma concentrations found in patients 12 h after conventional bleomycin chemotherapy (Slevin et al 1984).

Assuming that drinking water contains bleomycin at the highest concentration found in this study (13 ng L^{-1}), an individual drinking an average 2 L of water per day would ingest an amount of bleomycin equivalent to about one millionth of the normally prescribed daily dose for adult patients. The levels found in drinking water are therefore unlikely to present any risk to public health.

These data and those reported previously for methotrexate (Aherne et al 1985) indicate that levels of these two drugs in drinking water are extremely low. It is reasonable to assume that residues of other cytotoxic drugs are present at similar or lower levels because of their reactivity and biodegradability, and thus one may draw the general conclusion that residues of cytotoxic drugs which find their way into drinking water are unlikely to present any risk to public health.

The views contained in this paper are those of the authors and not necessarily those of the organizations they represent.

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