In the technique described here, fully conscious and unsedated guinea-pigs were lightly restrained in a perspex box inside an acoustic chamber. Skin electrodes were applied to the tragus and the vertex of the head. The pinna was earthed. Two thousand and forty-eight unfiltered clicks, having maximum energy in the 4 kHz range, were played to the animal from a TDH 39 earphone placed at a fixed distance in front of the head at a rate of 10 clicks/s, and at an intensity of 50 dB H.L.

The responses were recorded on a Medelec Mark 2 electro-cochleography apparatus, using filtration (0.25–5 kHz) common mode rejection and signal averaging to extract the signal from electrical noise.

In these circumstances, the responses to a click of fixed intensity are repeatable and stable over a period of 2 to 3 h and are sensitive to the administration of drugs which modify auditory function, either by physiological mechanisms or through ototoxicity.

This model has been used to investigate the efferent control of the ear using drugs which act upon cholinergic neurones.

The results of injecting atropine sulphate (250 μg/kg) depend on the initial values. When these are small and early, the tracings increase in amplitude as if the sound were some 30 to 50 db louder (a doubling of amplitude). The latency increases by up to 0.3 ms as if the sound were some 30 to 50 db quieter. When the responses are initially large and late, atropine has little effect.

Following the administration of physostigmine (0.2 mg/kg), amplitude decreases and latency increases. The effect of the administration of these drugs depends on the initial state of the system. These results support the view that acetyl choline is the efferent transmitter.

References

LEV, A. & SOHMER, H. (1972). Sources of averaged neural responses recorded in animal and human experiments during cochlear andiometry (electro-cochleogram). *Arch. klin. exp. Ohren Nasen Kehlkopfheilkd.*, **201**, 79–90.

SOHMER, H. & FEINMESSER, M. (1967). Cochlear action potentials recorded from the external ear in man. *Ann. Otol. Rhinol. Lar.*, **76**, 427–435.

A behavioural test applied in an acute toxicity screen

A.P. SILVERMAN

Department of Pharmacology, Materia Medica, and Therapeutics, University of Manchester, Manchester M13 9PT.

Behavioural toxicology will be most useful if it gives early warning of effects found later by conventional methods or finds effects at lower doses. Some years ago (Silverman, 1973) I briefly reported an 'exploration-thirst' test which seems informative and simple enough to investigate as a screen.

A rat explores a new environment. This is usually measured as motor activity but also implies curiosity, and it can also compete with other needs. Rats are deprived of water overnight and placed singly in an unfamiliar cage. Motor activity is scored as pauses/min when the rat stops walking to rear or sniff. The time is recorded (in s) until the rat finds water and begins to drink, if necessary distinguishing between the first lick and the first drink of at least 10 s: the interruption of drinking implies a need for more information about the cage. The rat is returned to the home cage after 5 min, offered water, and the procedure repeated next day.

As a first study of the method's usefulness in practice, it was added to the routine acute intraperitoneal toxicity test on 20 consecutive compounds. Survivors 6 h after injection were deprived of water unless they were obviously ill, and behaviour was observed at 24 and 48 hours.

Sixteen of the 20 compounds showed significant behavioural effects, in 11 at the lowest dose tested and in 12 where no effect was discovered clinically or at post-mortem. In 2 of the cases without behavioural effect, rats had recovered from obvious dyspnoea or convulsions.

Not surprisingly, a near-lethal dose often causes non-specific 'ILLS' (Increased Latency to drinking with Locomotion Slow). Effects on 1 day only indicated recovery or delayed onset. In 7 cases there was post-mortem evidence of peritonitis, at least at higher doses.

The use of two independent measures allows a more specific effect (presumably central) to be distinguished. This was not demonstrated in the present series, but in a subacute inhalation toxicity study, exposed rats walked twice as fast as controls without changing the latency to drinking. Rats exposed to trichloroethylene vapour (100–1000 ppm) drank earlier than controls while walking at the same rate (Silverman & Williams, 1975). Observation suggests other possible CNS causes of non-specific formal

results. In 4 of the present cases, rats alternated between immobile crouching and fast running, and another compound caused prolonged gnawing and grooming.

The effort involved averages about 0.25 man-hours/rat, perhaps 8 min actual observation and 7 min in preparation, data analysis and writing a routine report. This may be more than is justified in an acute screen but seems worthwhile at the subacute stage where early warning and tentative no-effect doses are most useful.

This work was done at Imperial Chemical Industries Ltd., Central Toxicology Laboratory, Alderley Park, Cheshire SK10 4TJ.

References

SILVERMAN, A.P. (1973). An 'Exploration-Thirst' test of chemical effects on behaviour. *Arch. Pharmac.*, **279**, *suppl.*, p. 25.

SILVERMAN, A.P. & WILLIAMS, H. (1975). Behaviour of rats exposed to trichloroethylene vapour. *Br. J. ind. Med.*, **32**, 308–315.

Comparison of the novel bibenzyl bifluranol with diethystilboestrol: effect of aromatic fluorine substitution on metabolism

R.P. CHAN, J.B. DEKANSKI, S. GOTTFRIED, D.V. PARKE & D.J. POPE

Biorex Laboratories Ltd., London N1 2HB, and Biochemistry Department, University of Surrey, Guildford GU2 5XH.

A novel compound, bifluranol, has been synthesized as part of a programme to examine the effects of various substituents on the endocrinological activity of bibenzyl structures related to hexoestrol (Figure 1). The bibenzyl structure was chosen in preference to the stilbene structure of diethylstilboestrol (DES) due to concern over DES toxicity. Orally bifluranol was shown to be a comparable antiandrogen to DES, but with only one-eighth of the oestrogenic activity, and is currently undergoing clinical evaluation for use in benign prostatic enlargement.

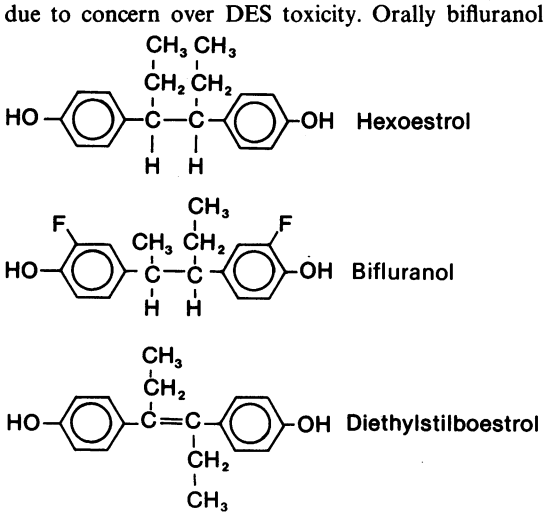

Figure 1 Structures of hexoestrol, bifluranol and diethylstilboestrol.

Studies on the fate of [^{3}H]-bifluranol have shown that the disposition in rat is similar to that reported for DES, with the drug being rapidly absorbed, taken up by the liver and eliminated in bile to give predominantly faecal excretion and low urinary excretion. However, the metabolic fate of the two compounds differs significantly. DES has been shown to undergo extensive oxidative metabolism in rat, with up to 30% being converted to a mixture of at least seven products including dienoestrol and hydroxy- and methoxy-derivatives of dienoestrol and DES (Metzler, 1976). The major biliary conjugate of DES is the monoglucuronide (75%), with small amounts of diglucuronide (10%) and polar products (15%) (Fischer, Millburn, Smith & Williams, 1966). In contrast bifluranol (2 mg/kg) undergoes oxidative metabolism only to the extent of 7% in rat to give a single main oxidation product. Mass spectrometry indicates that this metabolite has an additional hydroxyl group in one of the aromatic rings. The major biliary conjugates also differ, the monoglucuronide (50%) and glucuronide sulphate (42%) being the major products with small amounts of diglucuronide (8%). Further comparative studies are in progress to examine this lower extent of oxidative metabolism and the formation of a mixed double conjugate seen with bifluranol.

References

FISCHER, L.J., MILLBURN, P., SMITH, R.L. & WILLIAMS, R.T. (1966). The fate of ^{14}C-stilboestrol in the rat. *Biochem. J.*, **100** (3), 69P.

METZLER, M. (1976). Metabolic activation of carcinogenic diethylstilboestrol in rodents and humans. *J. Toxicol. Env. Hlth. Suppl.*, **1**, 21–35.