PARAQUAT | CORRESPONDENCE | 1995 →

RAILEX

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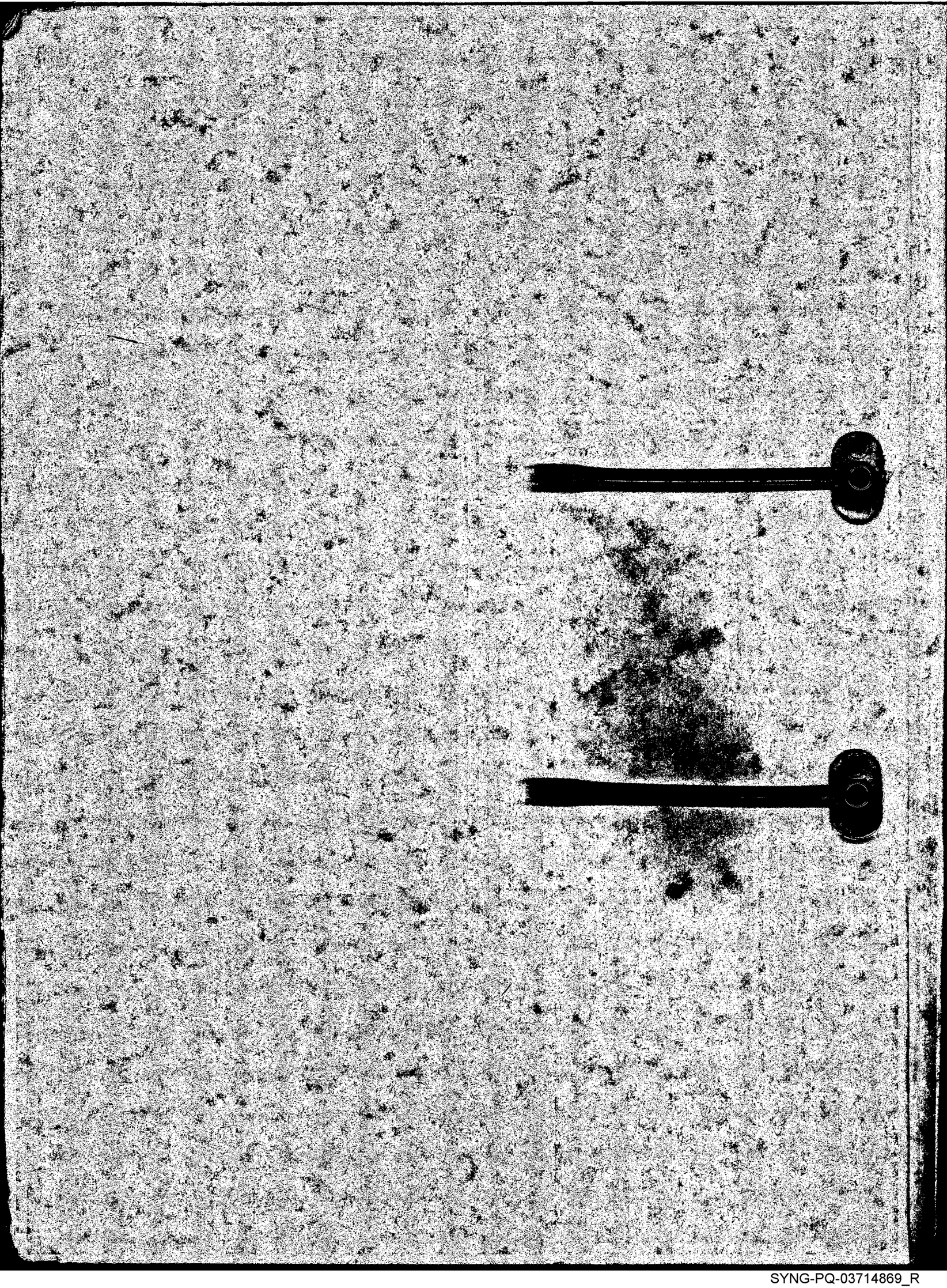

File : PQ Correspondence
PQ Formulation?

Internal Memorandum From
Andy Cook
Non-selective Herbicides Team Leader

ZENECA Agrochemicals
Regulatory Affairs Department
Fernhurst, Haslemere
Surrey GU27 3JE

Tel: Redacted - EU PII
Fax:
Z-Mail:
Telex: 858270 ZENAGR G

To
EU NC Registration Managers
EU NC Technical Managers
Diane Castle
Martyn Collins
Bob Kowalczyk
David Scott
Sid Shearing
Bob Scott
Jeremy Dyson/Mike Earl
Richa Shaunak
Alice Smith
Sally Baker

Copies

Our Ref	Your Ref	Ext	Date
		Redacted - EU PII	8 August 97

EU RESPONSIBILITIES FOR PARAQUAT

The current EU review programme, national product support, range rationalisation and new product development has resulted in an increased workload for the bipyridyls and in particular paraquat. It has therefore been necessary to re-distribute the existing resources within the WERAG Non-selective Herbicides Team on the basis of the importance of these compounds to the business.

The attached summary therefore clarifies these revised regulatory responsibilities within WERAG for both paraquat and diquat.

Please do hesitate to contact me if any further clarification is required.

Regards

Andy Cook
WERAG Non-selective Herbicides Team Leader

SUMMARY OF BIPYRIDYL RESPONSIBILITIES IN THE WERAG NON-SELECTIVE HERBICIDES TEAM

Andy Cook is the WERAG Non-Selective Herbicides Team Leader.

DIQUAT

Sonia Ellis is the EU Regulatory Manager for diquat.
Sonia Ellis handles all aspects of registration of diquat and diquat products within the EU (plus Norway).
(**Sonia Ellis** is also the international regulatory project focus for diquat)

PARAQUAT

Andy Cook is the EU Regulatory Manager for paraquat
(**Andy Cook** is also the international regulatory project focus for paraquat)

Andy Cook is reponsible for the regulatory strategy for paraquat and the EU review programme for paraquat, however additional paraquat regulatory support is provided by:

Jean Costello, responsible for current national registrations of all formulations containing paraquat alone or paraquat in mixture with OM products and for re-registration submissions (Annex III submissions) of these products post Annex I. This product list includes:

YF7697A ['Gramoxone 2' (Belgium), 'Gramoxone 100' (Ireland), 'Gramoxone W' (Italy), 'Gramoxone' (Netherlands), 'Gramoxone 2000' (Portugal), 'Gramoxone 100', 'Dextrone X', 'Speedway Liquid' (UK)]
['Gramoxone Extra N' (Spain)]
YF7949A ['Gramoxone' (tropics) (Greece)]
YF7362B ['Gramoxone Extra' (Germany)]
YF9845 ['Speeder', 'Gramixel 100', 'R-Bix', 'Gramix' (France)]
YF6939B pq/monolinuron mixture ['Gramonol A' (Ireland), 'Gramonol 5' (UK)]

Jean Costello should be your first point of contact for all regulatory issues on these formulations.

Jean Costello will also henceforth be the first point of contact for all regulatory issues relating to all (i.e. including glyphosate-trimesium & glyphosate) of the West European Non-selective Herbicide residue trial programmes.

Sonia Ellis, responsible for current national registrations of all formulations containing paraquat in mixture with diquat and for re-registration submissions (Annex III submissions) of these products post Annex I. This product list includes:

YF7779A pq/dq mixture ['Seccatutto' (Italy), 'Regal' (Greece)]
YF7768A pq/dq mixture ['Priglone' (Belgium), 'Farmon PDQ' (Ireland), 'Actor' (Netherlands), 'PDQ' (UK)]
pq.dq mixture ['Gramoxone Plus' (Spain)]
YF9835 pq/dq mixture ['Gramoxone Plus', France]
YF7439B pq/dq mixture ['Weedol' (Ireland), 'Weedol' (UK)]

Sonia Ellis should be your first point of contact for all regulatory issues on these formulations.

R:\RAD\WERAG\PARAQUAT\PQRESP.doc

ZENECA CENTRAL TOXICOLOGY LABORATORY
INVESTIGATIVE TOXICOLOGY SECTION
ALDERLEY PARK
MACCLESFIELD
CHESHIRE
SK10 4TJ

PARAQUAT ANALYSIS RESULTS

TO: Dr W D Neithercut, Chemical Pathology Dept., Arrowe Park Hosp., Arrowe Park Rd, Upton, Wirral, L49 5PE

COPIES TO: Dr R C Scott, Dr M F Wilks

FROM: Bruce Woollen

SAMPLE TYPE: Plasma, Serum and Urine (C/S No. 5017276)

SOURCE : Arrowe Park Hospital

ANALYST : D Blake

REFERENCE : Notebook 230227/8

SAMPLE TYPE	DATE OF SAMPLE COLLECTION	METHOD OF ANALYSIS	RESULT (paraquat ion ug/ml)
Sample 1 (Serum)	4.8.97 - 14.30	Radioimmunoassay	5.9
Sample 2 (Plasma)	4.8.97 - 17.50	Radioimmunoassay	1.6
Urine	4.8.97	Radioimmunoassay	100

COMMENTS :

Signed : ..... Position : Laboratory Manager, M & P Unit Dated : .....

CONTACT MR.B.H.WOOLLEN (TEL : 01625 515453)

ZENECA CENTRAL TOXICOLOGY LABORATORY
INVESTIGATIVE TOXICOLOGY SECTION
ALDERLEY PARK
MACCLESFIELD
CHESHIRE
SK10 4TJ

Dr Gen.

PARAQUAT ANALYSIS RESULTS

0151 706 2000

TO: Dr T. Hankin, Senior Registrar, Intensive Care Unit, Royal Liverpool University Hospitals, Prescot St., Liverpool, L7 8XP

COPIES TO: Dr R C Scott, Dr M F Wilks

FROM: Bruce Woollen

SAMPLE TYPE: Serum and Ultrafiltrate

SOURCE : Michael Morgan, Unit no. 2061311H

ANALYST : D Blake

REFERENCE : Notebook 230229/30

SAMPLE TYPE	DATE OF SAMPLE COLLECTION	METHOD OF ANALYSIS	RESULT (paraquat ion ug/ml)
Serum	7/8/97 12.30	Radioimmunoassay	0.33
Ultrafiltrate	7/8/97 12.30	Radioimmunoassay	0.31

COMMENTS :

Signed : B.H.Woollen Dated : 11/8/97

Laboratory Manager
Metabolism and Pharmacokinetics Unit

CONTACT MR.B.H.WOOLLEN (TEL : Redacted - EU PII)

WHILE YOU WERE OUT

Name Dr Guering

Of .....

Tel. Ext. Redacted - EU PII

Date 28/8/97 Time 2.10

Telephoned	☑	Please call him/her	☑
Called to see you	☐	Will call again	☐
Wanted to see you	☐	Urgent	☐

Message .....

.....

.....

Signed .....

Amphocil™ ZENECA

11056/Mar '94

11.15 | 28/8.

Bob

Dr. Guerin @ Redacted - EU PII has completed PM on Redacted - EU PII Redacted - EU PII and urgently requires our records so she can complete her report to the coroner today.

cl.

Telephone
Fax

Redacted - EU PII

TRANSMISSION REPORT

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:TCT ZENECA CTL (28 AUG '97 14:59)

DATE	START TIME	REMOTE TERMINAL IDENTIFICATION	MODE	TIME	RESULTS	TOTAL PAGES	DEPT. CODE	FILE NO.
28 AUG	14:58	751 706 5859	G3EST	00'56"	OK	02		

4/8/97. — Are there any other data

SYNG-PQ-03714877_R

ZENECA

Central Toxicology Laboratory
Alderley Park
Macclesfield
Cheshire SK10 4TJ
England

Telephone 01625 582711
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Fax 01625 585715

Fax Cover Sheet

To : DR GUERIN

Company Name : Redacted - EU PII

Fax Number :

From : Redacted - EU PII

Date : 29/8/97 Time :

Number of pages following cover note : 1

Cover Note: Dr Guerin –

Earlier paraquat sample results.

It appears that the first sample had a value of 5.9 (not 18) ug/ml.

Redacted - EU PII

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ZENECA

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Fax 01625 585715

Fax Cover Sheet

To : DR GUERIN

Company Name : Redacted - EU PII

Fax Number :

From : Redacted - EU PII

Date : 28/8/97 Time :

Number of pages following cover note : 1.

Cover Note:

Dr Guerin —

Copy of the paragraph results sent to Dr T Hanton.

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PQ - Corresp

6 - FEB 1997

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Dr M D Coleman
Aston University
Pharmaceutical & Biological Sciences
Aston Triangle
Birmingham B4 7ET

Our Ref	Your Ref	Direct Line	Ext	Date
MFW/jmh/L154D7.doc		Redacted - EU PII	Redacted - EU PII	4 Feb 97

Dear Dr Coleman

WR-2721

Thank you for your recent letter regarding the use of this radioprotectant drug. In fact, I was first contacted by Dr Vale last year but it took me quite a while to dig up some background information.

You will see from the above that I wasn't aware of the potential of this drug in the treatment of paraquat poisoning. One of the references, however, goes back to some work that was carried out in the 1980s at our Central Toxicology Laboratory (CTL). You will remember that it was at CTL that the active transport of paraquat into alveolar epithelial cells was first described, and, as part of that work, WR-2721 and some of its analogues were investigated because they are competitive inhibitors of the polyamine transport system.

This does open the possibility, at least in theory, of a dual action of this compound in paraquat poisoning. Firstly by competitive inhibition of the transport into alveolar cells, and secondly, by intracellular generation of free thiols which may protect against reactive oxygen species.

I am nevertheless sceptical about the usefulness of WR-2721 as a therapeutic agent in paraquat poisoning. It has a very short plasma half-life (around 2 minutes) which seems to be too short to allow selective accumulation in alveolar cells. Infusion of higher doses appears to be ruled out on grounds of cytotoxicity. Looking at the protection afforded against radiation treatment this amounts to a factor of only around 1.3 for lung and kidney. It is, of course, difficult to translate that for paraquat poisoning but we know that the majority of patients will have taken in more than 2-3 times a lethal dose of paraquat with suicidal intent and I would have thought that the protection would need to be much more effective to make a real difference. Last, but not least, the cost of such a treatment would make it probably impossible for health services in developing countries to afford.

I don't wish to sound too negative on this but you will appreciate that we are asked frequently to comment on various approaches to the treatment of paraquat poisoning. This one is an interesting idea, but I have many doubts about its practicalities. I have taken the liberty of copying our correspondence to colleagues at CTL for their opinion, and if they feel more positive than I do we should continue the dialogue.

Lastly, Zeneca does not actually market this drug, this is done by Schering-Plough.

Please let me know if you would like to discuss this further. I enclose a copy of the CTL paper for your reference.

With my best regards.

Yours sincerely,

Dr M F Wilks
Products Medical Adviser
Stewardship Department

cc. Mr Ciaran Kelly
Dr J A Vale, West Midlands Poisons Unit
Dr R C Scott, CTL

Encs.

INT. J. RADIAT. BIOL., 1989, VOL. 55, NO. 3, 463–472

Competition for polyamine uptake into rat lung slices by WR2721 and analogues

IAN WYATT, RICHARD B. MOORE and LEWIS L. SMITH

Biochemical Toxicology Section, ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, SK10 4TJ, U.K.

(Received 30 June 1988; revision received 5 October 1988; accepted 12 October 1988)

The objective of these studies was to determine whether a series of structurally related radioprotective agents could act as substrates for the recently identified polyamine system in the lung. We have shown that WR2721 (S-2(3-aminopropylamino)ethyl phosphorothioate), S-2(4-aminobutylamino)ethyl phosphorothioate (S-ABEP or WR2822) and S-2(7-aminoheptylamino)ethyl phosphorothioate (S-AHEP) competitively inhibit the uptake of putrescine into rat lung slices. The ability of the radioprotectors to act as substrates for the polyamine uptake system was expressed as the K_i for each compound. The K_i values for WR2721, S-ABEP and S-AHEP in the absence of dithiothreitol were 48, 57 and 7 μmol dm^{-3} compared to 155, 88 and 15 μmol dm^{-3} in the presence of dithiothreitol, indicating that the disulphide form may have a higher affinity for the transport system. By analogy with other substrates for the polyamine uptake system we have concluded that it should be possible to target radioprotectors to the alveolar epithelial type I and II cells and the Clara cells in the lung, as they prossess this uptake system, and thus protect these cells from oxidative stress.

1. Introduction

The polyamines putrescine, spermidine and spermine, and the herbicide paraquat are selectively accumulated into the rat lung by a common transport process which is energy-dependent and obeys saturation kinetics (Smith and Wyatt 1981, Smith *et al.* 1982, Rose *et al.* 1974). Both indirect (Smith and Wyatt 1981, Smith 1982, Sykes *et al.* 1977) and autoradiographical evidence (Nemery *et al.* 1987, Wyatt *et al.* 1988) have indicated that it is the alveolar epithelial type I and type II cells, and the Clara cell of the lung, that possess this transport process. The structural requirement for chemicals to act as substrates for this transport has been investigated using lung slices (Gordonsmith *et al.* 1983). A series of diamino-alkanes have been reported to inhibit the accumulation of putrescine into lung tissue and, therefore, by implication other polyamines and paraquat (Gordonsmith *et al.* 1983). The more effective diamine inhibitors were those with at least four methylene groups between the nitrogen atoms, and the maximum inhibitory potency was obtained when there were between seven and ten methylene groups (Gordonsmith *et al.* 1983). From these studies it was not possible to determine the optimum separation between the nitrogen atoms, since the diamino-alkanes do not have a rigid structure. However, the minimum separation that permits the molecule to act as an effective substrate for the uptake process is in excess of 0·5 nm (Gordonsmith *et al.* 1983).

In our search for other substrates for this transport process, we have studied the

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radioprotective compound WR2721 (S-2(3-aminopropylamino) ethyl phosphorothioate) because of its diamine-like structure. WR2721 is rapidly dephosphorylated *in vivo* by alkaline phosphatase (Tabachnik *et al.* 1982) to form the protective free-thiol, *N*-2-mercaptoethyl-1,3-diaminopropane (WR1065). The radioprotection given by WR2721 is in part attributed to the increased host tolerance afforded by the addition of the phosphate group to the thiol, and in part due to the formation of the free thiol with its ability to react with free radicals (Akerfeldt 1963, Yuhas and Phillips 1983). The separation of the nitrogen atmos in WR2721 by three methylene groups suggested that this structure should be a substrate (although relatively poor) for the polyamine transport process in the lung. Therefore, WR2721 may have the potential to be accumulated by the alveolar type I and type II epithelial cells and Clara cells, and in this way provide selective protection to these cell types.

Since WR2721 has only three methylene groups, we investigated two further radioprotective compounds (Yuhas and Phillips 1983, Yuhas *et al.* 1973) of similar structure containing four and seven methylene groups between the nitrogen atoms respectively (S-2(4-aminobutylamino)ethyl phosphorothioate (WR2822) and S-2(7-aminoheptylamino) ethyl phosphorothioate). We have used the ability of these compounds to inhibit the accumulation of putrescine into the lung as a possible indicator for the ability of these radioprotective agents to be themselves accumulated into the lung.

2. Materials and methods

2.1. *Materials*

[1,4-^{14}C]putrescine dihydrochloride (118 mCi/mmol) was purchased from Amersham International (Amersham, U.K.). Putrescine dihydrochloride, cysteamine hydrochloride and dithiothreitol (DTT) were supplied by Sigma Chemical Co (Poole, U.K.). WR2721 (S-2(3 aminopropylamino)ethyl phosphorothioate) was a gift from Drug Synthesis and Chemistry Branch, NC1, DHHS, Bethesda, Maryland, (U.S.A.). The compounds S-2(4-aminobutyl-amino)ethyl-phosphorothioate (S-ABEP or WR2822)) and S-2(7-aminoheptyl-amino)ethyl-phosphorothiate (S-AHEP) were synthesized according to published procedures (Piper *et al.* 1969). Soluene 350 (tissue solubilizer) and 'Dimilume' (scintillation cocktail) were purchased from Packard Ltd (Poole, U.K.). 'Optiphase' MP (scintillation cocktail) was supplied by LKB (Loughborough, U.K.). Halothane was obtained from ICI Pharmaceuticals (Macclesfield, U.K.).

2.2. *Animals*

Male Alderley Park Wistar derived specific pathogen-free rats (body weights approximately 200 g) were used throughout.

2.3. *Preparation of lung slices*

Rats were killed with halothane, the lungs removed and slices 0·5 mm thick prepared using a McIlwain tissue chopper.

2.4. *Uptake of [^{14}C]putrescine*

Freshly prepared lung slices (20–40 mg) were incubated in 3·0 ml of modified Krebs-Ringer phosphate (KRP) containing NaCl (130 mmol dm^{-3}), KCl (5·2 mmol dm^{-3}), $CaCl_2$ (1·9 mmol dm^{-3}), $MgSO_4$ (1·29 mmol dm^{-3}), Na_2HPO_4 pH 7·4 (10 mmol dm^{-3}) and glucose (11 mmol dm^{-3}) in the presence of 2, 5, 10, 20 or 50 μmol dm^{-3} [^{14}C]putrescine (0·3 μmol dm^{-3} [^{14}C]putrescine plus the required

amount of unlabelled putrescine) with or without 10, 25 or 100 μmol dm^{-3} of WR2721, S-ABEP (WR2822) or S-AHEP for 30 min at 37°C. Each study was performed in triplicate with one animal supplying enough lung slices for the comparison of control accumulation with that of the accumulation in the presence of the three treatment concentrations for one compound.

2.5. *The effect of dithiothreitol on uptake*

The incubations were performed with the same [^{14}C]putrescine concentrations and conditions as above, in the presence or absence of 1 mmol dm^{-3} DTT with or without 100 μmol dm^{-3} WR2721, 50 μmol dm^{-3} S-ABEP (WR2822), 30 μmol dm^{-3} cysteamine or 10 μmol dm^{-3} S-AHEP.

2.6. *Determination of putrescine accumulation by the lung slice*

At the end of the incubation period, lung slices were removed from the incubation medium, washed with KRP, dissolved in 1 ml Soluene and 10 ml of Dimilume added. The level of radiolabel in the lung slice and medium was determined on a liquid scintillation spectrometer. The concentration of putrescine accumulated in the lung slice was calculated using the specific activity of the putrescine in the incubation medium, since lung slices have been shown previously not to metabolize putrescine (Smith and Wyatt 1981). Michaelis constants (k_m) and inhibitor constants (K_i) were determined graphically, as described by Dixon and Webb (1960), using a lineweaver–Burk plot (the reciprocal of the rate of accumulation (v) against the reciprocal of the substrate concentration (c)). The rate of putrescine accumulation (v) in the lung slice was taken as the amount of putrescine accumulated after 30 min, since it has previously been demonstrated that the accumulation into lung slices is linear over 30 min, for the concentrations studied (Smith and Wyatt 1981, Smith *et al.* 1982).

3. Results and discussion

In these studies we have used the ability of the S-2(aminoalkylamino)ethyl phosphorothioates to competitively inhibit the uptake of putrescine into the lung as a possible indicator for the ability of these radioprotectors to be themselves accumulated into the lung. This rationale is based on an extensive database in our laboratory which has shown that chemicals that competitively inhibit the uptake of putrescine are themselves accumulated into the lung. Furthermore, other authors (Rannels *et al.* 1985, Rannels and Addison 1987) have described the competitive inhibition of polyamine uptake in the lung by the anti-leukaemic drug methylglyoxal bis(guanylhydrazone) (MGBG), and shown that MGBG itself is a substrate for the lung uptake system. We know of no exceptions to this relationship, although these observations could obviously be extended by the use of radiolabelled radioprotectors.

All three radioprotective agents competitively inhibited the accumulation of putrescine into rat lung slices (figure 1). Their inhibitory potency tended to increase as the number of methylene groups between the nitrogen atoms increased (table 1). The apparent K_m for putrescine accumulation into lung slices was found to be 15 μmol dm^{-3} with a V_{max} of approximately 1000 nmol(g wet wt)$^{-1}$h^{-1} in agreement with our previously reported values (Smith *et al.* 1982). The K_i values determined for WR2721, S-ABEP (WR2822) and S-AHEP were 48, 57 and 7 μmol dm^{-3} respectively. Given that the inhibition of putrescine accumulation was

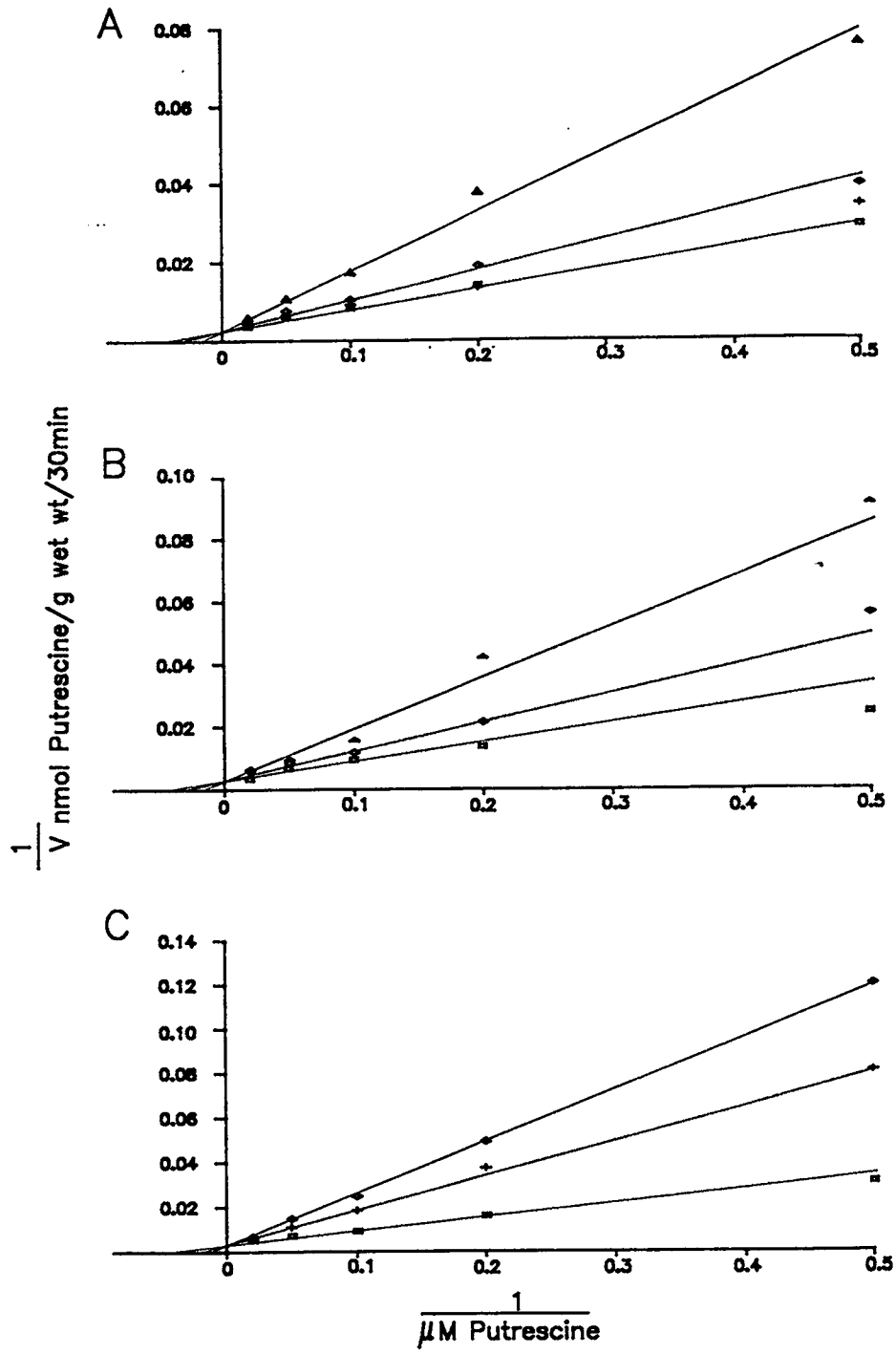

Figure 1. Lineweaver–Burk plot of [^{14}C]putrescine uptake by lung slices in the presence of (**A**) WR2721, (**B**) S-ABEP, (**C**) S-AHEP. □, Control, +, 10 μmol dm^{-3}, ◇ 25 μmol dm^{-3}, △ 100 μmol dm^{-3}. Each point was the mean of three observations.

Table 1. The inhibitory constant (K_i) for the inhibition of putrescine accumulation into lung slices by the S-2-(aminoalkylamino)ethyl phosphorothioate in the presence (+) or absence (−) of dithiothreitol DTT.

Compound	Structure	DTT	K_i(μmol dm^{-3})
WR2721	$H_2N(CH_2)_3NH(CH_2)_2$ SPO_3H_2	−	48
		+	155
S-ABEP (WR2822)	$H_2N(CH_2)_4NH(CH_2)_2$ SPO_3H_2	−	57
		+	88
S-AHEP	$H_2N(CH_2)_7NH(CH_2)_2$ SPO_3H_2	−	7
		+	15
Cysteamine	$H_2N(CH_2)_2SH$	−	11
		+	no inhibition

The inhibitory constants were derived from the Lineweaver-Burk plots of [^{14}C]putrescine uptake by lung slices in the presence of 100 μmol dm^{-3} WR2721, 50 μmol dm^{-3} S-ABEP, 30 μmol dm^{-3} cysteamine or 10 μmol dm^{-3} S-AHEP in the prescence or absence of DTT. The inhibitory constants were determined using the method of Dixon and Webb (1960) for competitive inhibition. The linear regression lines for all the Lineweaver–Burk plots had a correlation coefficient of greater than 0·9.

competitive (figure 1) it can be argued that the K_m for the uptake of these chemicals would be 48, 57 and 7 μmol dm^{-3} respectively for a fully competitive inhibitor. Examples of this logic are already published for polyamine transport in the lung. Rannels and Addison (1987) demonstrated that the apparent K_m for spermidine uptake in the lung was 1·7 μmol dm^{-3}, whilst Rannels *et al.* (1985) had a K_i (for spermidine) of 1·9 μmol dm^{-3} for the competitive inhibition of MGBG uptake into the lung. Also, using lung slices, the apparent K_m for MGBG accumulation was 7 μmol dm^{-3} (Gordonsmith *et al.* 1985) and MGBG competitively inhibits the accumulation of putrescine into lung slices with a K_i of 3·5 μmol dm^{-3} (I. Wyatt, unpublished observations).

WR2721 can be dephosphorylated to the free thiol by alkaline phosphatase (Tabachnik *et al.* 1982), an enzyme known to be localized in the alveolar epithelial type II cells (Fisher *et al.* 1980). Previous studies in our laboratory have shown that cysteamine ($H_2N(CH_2)_2SH$) is rapidly converted to the disulphide cystamine ($H_2N(CH_2)_2$-S-S-$(CH_2)_2NH_2$) under the conditions used for investigating accumulation into lung slices (data not presented). This oxidation of cysteamine is prevented if the incubations are carried out in the presence of dithiothreitol (DTT). Cysteamine in the presence of DTT does not block the accumulation of putrescine (table 1), whereas in the absence of DTT (when oxidized to cystamine) it had a K_i of 11 μmol dm^{-3} (table 1). By analogy with cysteamine, oxidation to the disulphide may occur with the S-2(aminoalkylamino)ethyl phosphorothioates when they are incubated in the medium with lung slices. Therefore the inhibitory effect of these compounds on putrescine uptake into lung was studied in the presence and absence of DTT. The effect of DTT on the ability of WR2721 to inhibit putrescine is shown in figure 2. The apparent K_i was shifted from 48 μmol dm^{-3} in the absence of DTT to 155 μmol dm^{-3} in its presence (table 1). In both cases the inhibition was

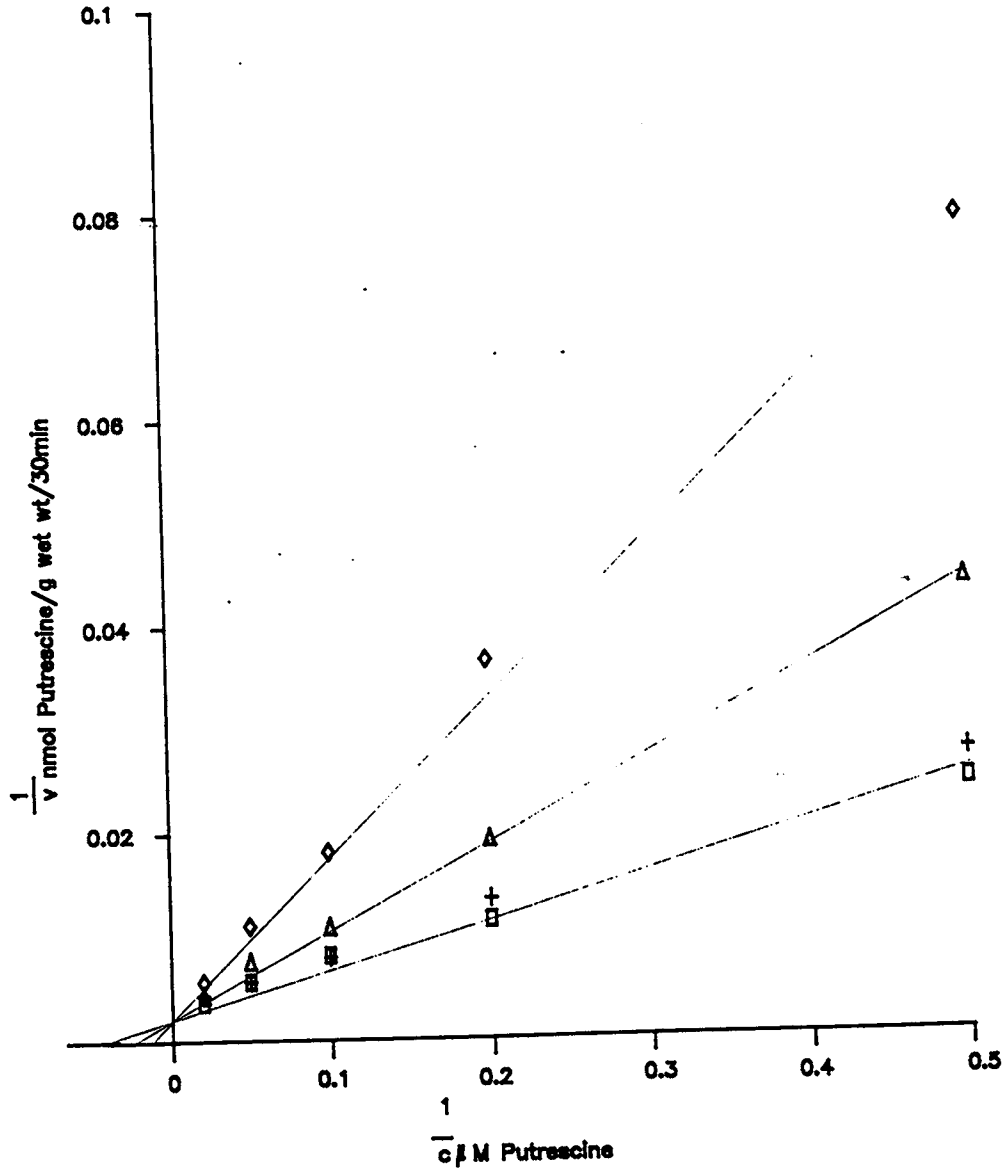

Figure 2. Lineweaver–Burk plot of [^{14}C]putrescine uptake by lung slices in the presence of 100 μmol dm^{-3} WR2721 plus or minus DTT. □, Control, +, plus 1 mmol dm^{-3} DTT, △ 100 μmol dm^{-3} WR2721 + 1 mmol dm^{-3} DTT, ◇ 100 mmol dm^{-3} WR2721. Each point was the mean of three observations.

found to be competitive (figure 2). Also, the addition of 1 mmol dm^{-3} DTT to the incubation medium had no effect on the accumulation of putrescine into the lung slice (figure 2). Similarly the K_i of S-ABEP (WR2822) was increased from 57 μmol dm^{-3} to 88 μmol dm^{-3} and K_i for S-AHEP from 7 μmol dm^{-3} to 15 μmol dm^{-3} (table 1).

These results suggest that under our experimental conditions the S-2(amino-alkylamino)ethyl phosphorothioates could be dephosphorylated by alkaline phosphatase from the lung slice to the free thiol, as suggested for WR2721 *in vivo* (Tabachnik *et al.* 1982). The free thiols are spontaneously oxidized to the disulphide in the incubation medium as occurs with cysteamine (data not presented). This hypothesis is depicted in figure 3. Consequently, when the radioprotectors are in the disulphide form they are more effective at competing for the uptake site. In the presence of DTT the compounds are held as free thiols, and in this form cannot compete as effectively for the transport site (table 1). It is interesting to compare these results with those obtained by Gordonsmith *et al.* 1983, who showed that the I_{50} for diaminopropane against putrescine was $124\,\mu mol\,dm^{-3}$ and that for the diaminoheptane was $6\,\mu mol\,dm^{-3}$, when the substrate concentration was $1\,\mu mol\,dm^{-3}$. The results for WR2721 and S-AHEP in the presence of DTT are very similar (K_i 155 and $15\,\mu mol\,dm^{-3}$ respectively). This suggests that the transport receptor could recognize the diamine structure in either the free-thiol or disulphide form. However, in the disulphide form the chemicals act as much more effective inhibitors of putrescine accumulation, and by analogy will be more effectively accumulated by the lung themselves.

Our results suggest that it may be possible to target chemicals with the potential to increase thiol levels in the alveolar epithelial type I and type II cells and Clara cells of the lung. This could be achieved with either radioprotectors in the free-thiol state or in the disulphide form. Furthermore, it is clear by altering the number of methylene groups separating the amino groups in the radioprotectors, that it is possible to alter the affinity of the chemical for the uptake process. These results have all been obtained using lung slices *in vitro*. When the accumulation of the herbicide paraquat was first described in lung slices we were able to show that it was similarly accumulated by the lung *in vivo* (Smith *et al.* 1974). Also, we have recently demonstrated that the diamine, putrescine, is also accumulated by the isolated perfused lung and the lung *in vivo* (Wyatt *et al.* 1988). It seems reasonable to argue therefore that these radioprotectors may also be accumulated into the lung *in vivo*. However, the kinetics associated with this transport process suggests that it is important to maintain the substrate in the plasma for prolonged periods of time in order that the lung can accumulate these compounds selectively (Smith *et al.* 1974). We know that, in the case of paraquat, following p.o. dosing to rats, it takes approximately 30 h of relatively constant concentrations of paraquat in the plasma to achieve concentrations in the lung 7 times that found in the plasma (Smith *et al.* 1974). Also, with putrescine uptake into the lung, it takes many hours before the lung accumulates concentrations in excess of the plasma (Wyatt *et al.* 1988). In order to optimize the 'targeting' of radioprotectors to specific lung cells (alveolar epithelial type I and type II cells and Clara cells), it may be necessary to administer the compounds in a manner that provides the plasma with substrate concentrations that are maintained constant for many hours and are in the range of the apparent K_m for the transport process. However, protecting normal lung cells as opposed to neoplastic cells may depend on the absence of the polyamine uptake system in the tumour tissue.

This suggestion is in contrast to the administration of WR2721 as an infusion to patients over 15 min just prior to radiation or chemotherapy treatment for malignancy (Glover *et al.* 1986). The clearance of WR2721 from the plasma is

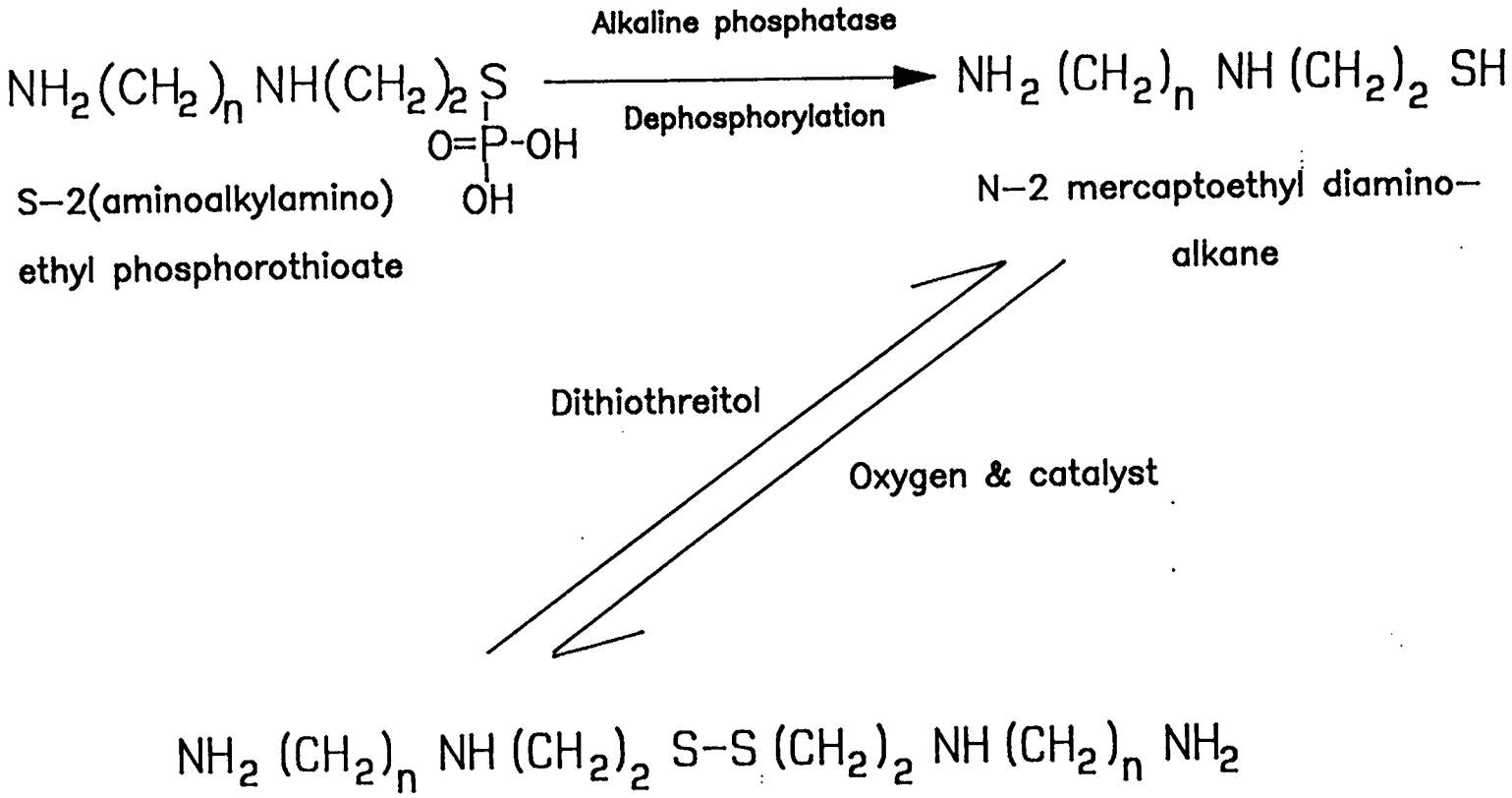

Figure 3. Proposed molecular transformations during incubation.

claimed to be rapid following infusion over a short time, with the majority of the WR2721 being cleared from the plasma within 10 min following a 10 s infusion of 150 mg/m^2 (Shaw *et al.* 1986). However, this bolus dosing of WR2721 will not make use of the transport process in the specific lung cells, and is therefore unlikely to achieve the best therapeutic index for the lung during radiotherapy treatment. Moreover, if this is correct, WR2721 does not have the optimum molecular structure to utilize the transport process, S-ABEP (WR2822) and S-AHEP having a higher affinity than WR2721 for the transport system.

The lung is not the only organ that has the ability to accumulate polyamines. Polyamine accumulation has been found in the brain (Smith *et al.* 1982); salivary glands, seminal vesicles, non-transformed ADIII white cells, and murine leukaemic WEHI III white cells (Smith *et al.* 1986); prostatic cancer cells (Heston *et al.* 1987); neuroblastoma cells (Rinehart and Chen 1984); and ascites L1210 leukaemic cells (Porter *et al.* 1985). Theoretically, it should be possible to target sulphur-containing chemicals to these cell types so as to alter the thiol status, provided they are accumulated by the polyamine transport process and thus protect the cells from oxidative stress. This principle will apply to a diverse range of substrates that meet the structural requirements for the polyamine uptake system, and offers the possibility of designing cytotoxic drugs that will be selectively accumulated by cancer cell types that possess the polyamine uptake system.

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File : PQ(Correspondence

Bob.
Copies of the abstracts as promised at PT meeting.
Ted

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Telephone:
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25 SEP 1996

Reference:

Dr EA Lock,
Central Toxicology Laboratory,
Alderley Park,
Macclesfield,
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England.

Dear Ted,

I have finally submitted my PhD thesis in April this year and 4 of the 6 papers on paraquat have been accepted for publication by various journals (The effect of paraquat on two renal epithelial cell lines - LLC-PK_1 and MDCK by *Research Communications in Pharmacology and Toxicology*, The mechanisms of excretion of paraquat in rats by *Toxicology Letters*, Transport of paraquat in a renal epithelial cell line LLC-PK_1 by *Journal of Pharmacology and Experimental Therapeutics*, Characterisation and uptake of paraquat by rat renal proximal tubular cells in primary culture by *Human and Experimental Toxicology*). The papers are enclosed.

Now we would like to validate our results using a human proximal renal tubular cell line HK2 and find out if cations protect against the nephrotoxic effect of paraquat. However, we have run out of [^{3}H] paraquat and have very little [^{14}C] paraquat left. I would really appreciate if you could consider providing us with more [^{3}H]paraquat so that we may continue our studies on the renal excretory mechanisms and transport of paraquat. It would be very much appreciated if you could help us out again. Geoffrey Duggin asked me to send his warmest regards to you. Thanking you for your assistance.

Yours sincerely,

Betty Chan
30/8/96

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Research Communications in
Pharmacology and Toxicology

THE EFFECT OF PARAQUAT ON TWO RENAL EPITHELIAL CELL LINES - LLC-PK$_1$ AND MDCK

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2: Electron Microscopy Unit, Royal Prince Alfred Hospital.

3: Dept of Pharmacology, Bosch Building, Sydney University, 2006, NSW, Australia.

Abstract

The effects of paraquat (PQ) were determined on two renal epithelial cell lines which resemble proximal (LLC-PK$_1$) and distal tubular cells (MDCK), using studies of cellular viability and cytotoxicity. PQ was found to have significant time and dose-dependent effects on cellular viability, cellular regeneration, DNA and protein synthesis ($p<0.0001$) and degenerative changes under electron microscopy on the LLC-PK$_1$ cells. Cellular regeneration of the LLC-PK$_1$ cells was evident only at the lower concentrations of PQ with a LC_{50} of 50 μM at 24 hour incubation. MDCK cells were found to be significantly more resistant to the effects of PQ by cellular viability studies, DNA and protein synthesis when compared with LLC-PK$_1$ cells ($p<0.0001$). The LC_{50} for LLC-PK$_1$ and MDCK cells were 24 and 417 μM for trypan blue exclusion, 5 and 360 μM for DNA synthesis, 67 and 680 μM for protein synthesis respectively at 24 hour incubation with PQ. These findings suggest that the LLC-PK$_1$ is more sensitive to the toxic effects of PQ when compared with the MDCK cell line.

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TITLE: THE MECHANISM OF EXCRETION OF PARAQUAT IN RATS

AUTHORS: Chan B. S. H.[1], Seale J. P.[2], Duggin G. G.[1,2].

[1] Dept of Renal Medicine and Toxicology Unit, Royal Prince Alfred Hospital

[2] Dept of Pharmacology, University of Sydney, 2006.

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Abstract

Paraquat (PQ) (1,1' dimethyl -4,4'-bipyridinium) is a toxic herbicidal cation. The renal excretory mechanisms of PQ and its interactions with organic cations and anions were investigated in anaesthetised rats. The renal clearance of PQ was studied in male Wistar rats using inulin as the marker of glomerular filtration rate. The fractional excretion of paraquat (FEpq) decreased from 2.1 ± 0.01 to 1.2 ± 0.03 as the plasma concentration rose from 0.4 ± 0.02 to 21.2 ± 1.6 μM. These results demonstrated that the excretion of PQ was greater than glomerular filtration, concentration dependent and saturable, indicating that it was secreted by an active transport system. The excretion of PQ was dependent predominantly on glomerular filtration rate with a small secretory component (Km = 8.5 ± 3.1 μM, Vmax = 114 ± 19 nmol/kg/min). The clearance of PQ was not inhibited by high doses of cimetidine, or *p*-aminohippurate However, quinine (p = 0.001) and N-methylnicotinamide (NMN) (p = 0.03) reduced the FEpq, suggesting that they share a similar cation transport system with PQ. In summary, PQ is actively secreted by the rat kidney via a cation transport system.

Key words: Paraquat, renal clearance, cation.

TRANSPORT OF PARAQUAT IN A RENAL EPITHELIAL CELL LINE LLC-PK_1

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LABORATORY IN ORIGIN: Renal Laboratory, Royal Prince Alfred Hospital, Missenden Rd, Camperdown, NSW, 2050, Australia.

Abstract

Transport of paraquat (PQ), a cationic herbicide, was investigated in a proximal renal epithelial cell line, LLC-PK_1. Collagen coated permeable filters were used to study the direction of PQ transport. PQ was transported predominantly from the basolateral to apical (B→A) membrane of these cells. The B→A flux and uptake of PQ were saturable with time and increasing concentrations, energy dependent and inhibited by a number of cations. Quinine was the most potent inhibitor of basolateral PQ uptake, followed by cimetidine, and then tetraethyl-ammonium acetate ($P < 0.0001$). The non-inhibitable basolateral uptake of PQ has an apparent Km of 357 μ M and Vmax of 1.47 pmol/μg protein/2 min. For flux studies, only quinine inhibited the B→A flux of PQ ($P = 0.02$). Putrescine, *p*-aminohippurate (PAH), probenecid, N-methylnicotinamide (NMN) and choline did not inhibit the flux or uptake of PQ. 5 N,N-hexamethylene amiloride (HMA), a cationic amiloride analogue and a potent inhibitor of the Na/H exchanger, significantly inhibited the uptake of PQ from either side ($P < 0.0001$). Acidic pH in the apical medium inhibited the uptake of PQ from either side. The studies demonstrated that PQ was actively transported by the LLC-PK_1 cells. PQ shared a similar transport system with a number of the cations, which appeared to have a more significant inhibition on the transcellular uptake than the flux of PQ.

Characterisation and Uptake of Paraquat by Rat Renal Proximal Tubular Cells in Primary Culture

AUTHORS: Chan B. S. H.[1,2], Lazzaro V. A.[1], Seale J. P.[2], Duggin G. G.[1,2].

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An abbreviated title: Characterisation and uptake of PQ by rat PTC in primary culture.

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Summary

1. Uptake of the herbicide paraquat (PQ), by rat proximal tubular cells (PTC) in primary culture grown on a collagen coated support was investigated.

2. The uptake of PQ by PTC was predominantly from the basolateral side. The basolateral uptake of PQ was saturable with time and increasing concentrations, energy dependent and inhibited by some organic cations. Using Michaelis Menten kinetics, the apparent Km was 778 ± 241 μM and Vmax was 0.97 ± 0.24 pmol/μg protein/15 min for the basolateral uptake of PQ. Cimetidine (5.7 ± 0.4 pg/μg protein/30 min, $p < 0.001$) was the most potent inhibitor of PQ uptake, followed by quinine (6.5 ± 0.4 pg/μg protein/30 min, $p < 0.01$) and then tetraethylammonium (8.2 ± 0.5 pg/μg protein/30 min, $p < 0.05$) when compared with control (11 ± 1 pg/μg protein/30 min). N-methylnicotinamide, *p*-aminohippurate and putrescine did not inhibit the basolateral uptake of PQ. The sodium hydrogen exchange inhibitors, amiloride and its analogue, 5 (N, N hexamethylene) amiloride (HMA) inhibited both apical and basolateral uptake of PQ.

3. The apical uptake of PQ was not saturable with increasing concentrations and was not inhibited by 2, 4-dinitrophenol, but it was reduced by cimetidine ($p < 0.01$), quinine ($p < 0.05$) and a sodium potassium ATPase inhibitor, ouabain ($p < 0.01$).

4. It is concluded that PQ was taken up from the basolateral side of primary cultured rat PTC by an energy dependent transport system.

Keywords: Paraquat, characterisation, uptake, renal proximal tubular cells, rat.

PQ/CORRESP.

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To DR M.B. WOODNER

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From DR R C Scott

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WE WISH YOU GOOD LUCK WITH YOUR STUDIES AND WILL BE PLEASED IF YOU WOULD ALLOW US TO SEE YOUR DATA, WHEN AVAILABLE.

PLEASE KEEP IN CONTACT WITH ME.

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FROM Dr M.B WOODNUTT **DATE** : 19/9/96

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ORGANISATION :

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DR. WOODNUTT / OLIVE EDWARDS

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Centre for Environmental Health Engineering
Department of Civil Eng. University of Surrey, Guildford, Surrey GU2 5XH

University of Surrey

FAX TO: 01625 585715
Attention: Mr Bob Scot
ICI
Macclesfield,
UK

From: Dr Million B. Woudneh
Fax: 01483 450984
Tel: Redacted - EU PII

19 September 1996

Dear Mr Bob Scot,

Re: Request for 1,1'-Diethyl-4-4'-bibyridylium diiodide

Following your request for a protocol of our research in St. Lucia, I have tried to write a short summary of the overall objectives of the project in the context of the requirements of the chemical which we request from your company.

Research Title

The development and integration of biotic and chemical monitoring with land use assessment for tropical river resource management.

Sponsor

The Overseas Development Administration

Broad objectives

1) To undertake intensive baseline biological and chemical survey of rivers in St. Lucia.

2) To identify the principal point and non point sources of pollution which give rise to the key pollution problems.

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Eur Ing Professor G R I Clayton
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DEPARTMENT OF
CIVIL ENGINEERING

University of Surrey

3) To asses the influence of defined physical and chemical pollutants on the biotic indices so as to identify the key causes of biotic damage.

4) To integrate water quality and geographic data and identify the extent to which human activities are causing measurable deterioration in quality.

5)To identify high conflict land use areas as a priority for intervention and management.

In the context of these wide objectives, paraquat is one of the most heavily used herbicides for the banana plantations regions. We therefore found the monitoring of this herbicide a priority in the recent water quality objectives of the area. The analysis of this herbicide requires a closely related analogue of the herbicide, 1,1'-diethyl-4,4'-biparidilium diiodide, which is available in your company only. We therefore request your kindest cooperation to enable us to meet one of our objectives.

I would also like to stress the fact that we would be happy to send you a copy of our findings should you be interested in this.

Best wishes.

Dr M. B. Woudneh.

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Centre for Environmental Health Engineering
Department of Civil Eng. University of Surrey, Guildford, Surrey GU2 5XH

University of Surrey

FAX TO: Redacted - EU PII
Attention: Mr Mike Crowley
ICI
Macclesfield,
UK

From: Dr Million B. Woudneh
Fax: 01483 450984
Tel: Redacted - EU PII

Dear Mr Crowley,

Re: Request for 1,1'-Diethyl-4-4'-bibyridylium diiodide

Currently our department is planning to undergo a paraquat monitoring program in one of the Caribbean islands, St Lucia. The monitoring system requires the use of an internal standard which to the best of our knowledge is available only in your department. I am therefore requesting if it is possible for you to supply us with the above chemical so that we will be able to undertake the environmental monitoring of paraquat in St. Lucia.

Since the purpose of the chemical is for use as an internal standard I think an amount of 1 - 2 g is more than sufficient to our purpose.

Thank you for your cooperation.

Dr M.B. Woudneh

Department of Civil Engineering
University of Surrey
Guildford Surrey GU2 5XH England
Telephone: +44 (0)1483 300800
Fax: +44 (0)1483 450984 Telex: 859331

Eur Ing Professor C R I Clayton
MSc DIC PhD CEng FICE CGeol FGS
Head of Department
Professor of Geotechnical Engineering

DEPARTMENT OF
CIVIL ENGINEERING

B/F
16/9
BDT

Bob

Spoke to this guy on Friday.

Thought 9'd better.

Dear Ashdown

Redacted - EU PII

St Lucian Forces staff currently Qwar Wandsey.

Would be good PR to provide standards.

PQ St Lucian internal. Standards: yes? details.

call Dan for

Mike.

Redacted - EU PII

B/F
16/9
BDT

Bob

Spoke to this guy on Friday.

Thought I'd better ask you, before sending a sample

Written to Dan Ashdown

Mike.

Redacted - EU PII

BDr

WHILE YOU WERE OUT

Name Dr. Woundneh.

Of University of Surrey.

Tel. Ext. Redacted - EU PII

Date 18/9 Time 14-40

Telephoned	☑	Please call him/her	☑
Called to see you	☐	Will call again	☐
Wanted to see you	☐	Urgent	☑

Message re samples.
have you a response
for him please

Signed

ZENECA

Confronting the Challenge of Infection

11056/Mar '94

ZENECA

Central Toxicology Laboratory
Alderley Park
Macclesfield
Cheshire SK10 4TJ
England

Telephone 01625 582711
Telex 669095/669388 ZENPHA G
Fax 01625 585715

Fax Cover Sheet

To DR M B Woudneh

Company Name Unverty of Surrey

Fax No. 01483 509844

From DR R C Scott

Date 16th Sept '96 **Time**

No. of pages following cover note

Cover Note Request for 1'1'-diethyl-4-4' bipyridylium dichloride

I am reviewing your request for this analytical standard.
I am afraid I am unaware of this proposed study.
Will you please provide details and your protocol to allow me to consider your request further and assist your work.

Yours
Bob Scott, Paraquat Product Toxicologist.

Please confirm receipt as soon as possible to Telex 669095 ZENPHA G or via internal Zeneca Network PHNAZEN

In the event of poor transmission:
Non - Zeneca please ring Redacted - EU PII

AP3269CTL6

Central Toxicology Laboratory in the UK is part of Zeneca Limited.
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Registered Office
15 Stanhope Gate
London W1Y 6LN

Internal Memorandum From
Samantha Evans
Regulatory Officer

ZENECA Agrochemicals
Regulatory Affairs Department
Fernhurst, Haslemere
Surrey GU27 3JE

Tel: Redacted - EU PII
Fax: 01428 655949
Z-Mail:
Telex: 858270 ZENAGR G

To
BobScott
Mike Clapp
Jon Heylings
Mike Simpson
Sue Jones
Neil Wilson

Copies

Our Ref	Your Ref	Ext	Date
		Redacted - EU PII	16 Oct 95

RECENT VISIT TO THE UK BY CARLOS CAJAS, ZENECA PANAMERICANA

Just a short note on behalf of Carlos Cajas to thank you all very much for the time that you gave to both myself and Carlos during his recent visit to the UK.

He found all of the presentations and information given to him during his visit very interesting and I am sure he will put his new found knowledge to good use on his return to Central America.

We would particularly like to thank Bob Scott for putting together such an excellent programme at such short notice.

I look forward to seeing you all again sometime in the future.

Thank-you all again

Samantha

To. Bob Scott for information

cc. A Wheeler
D. Castle

24 MAY 1995

PQ — Correspondence
— Denmark
— Neurotox

K H Vestergaard
Zeneca Agro
Islands Brygge 41
2300 Kobenhavn S
Denmark

Your ref Our ref Direct line Redacted - EU PII Tel ext Redacted - EU PII Date 18-May-1995

Dear Karl Henrik

SWEDISH NEUROTOXICOLOGY RESEARCH INVOLVING PYRETHROIDS

Thank you for your recent letter requesting some background information on the toxicology studies conducted by Per Eriksson's group at Uppsala, and for the information on the DEPA report on neurotoxicity. I will try and give you the information you require as concise as possible.

First and most easily it would be useful to have the DEPA report in CTL, could you please send me a copy.

Now to the Eriksson studies, thank you for the translation of the Swedish newspaper report, I had heard it was in the headlines as I attended a meeting on pyrethroids at Oxford University in March and Dr Kolmodin-Hedman, from Huddinge University Hospital, Stockholm gave a paper at the meeting on her experience with forestry workers handling pyrethroids and mentioned it had been in the news.

Per Eriksson's work and experience is all with **mice**, the article has extrapolated to children. However as the report mentions he has been looking at the effect of a range of pesticides and persistent chemicals (pyrethroids, OP's and paraquat of particular interest to Zeneca) when given to very young mice, typically around 10-11days old, at a time when the brain is undergoing its maximum growth spurt. This is potentially a very vulnerable period when any "toxic" insult might have a longer term effect on the animals behaviour.

Based on this premise Eriksson has been conducting studies in this area for at least 10 years, his first paper on a pyrethroid was reported in 1990! I have enclose a list of some of his papers for your use and photocopies of the key ones on bioallethrin, deltamethrin and paraquat. The design of his experiments is quite simple, and has two end points. One looking for behavioural changes in the mice, soon after dosing and them typically 60 and 120 days latter. Two he makes some neurochemical measurements, for example with paraquat the concentration of certain neurotransmitters in the brain and with the pyrethroids the density of muscarinic receptors in the brain at the end of the study.

He uses, as the article points out very low oral doses of the chemicals. For example with paraquat 0.07 and 0.35mg/kg on days 10 and 11 of age and reports

that at 60 and 120 days of age the mice are less active than those not given paraquat. At 125 days the mice were killed and measurement made on the brain and decreases in dopamine and its metabolites found compared with controls. In addition, he compared paraquat with MPTP and found a similar response. This was what brought Eriksson's work to my attention, as the doses are many fold below our no-effect levels from all our other toxicology end points, and about 500 to 2600 times lower than the median lethal dose(MLD) in mice. MLD in mice given paraquat orally is about 200mg/kg. Plus the link with MPTP and Parkinson's disease.

The pyrethroids studies were similar except the dosing was typically daily from day 10 to 16 inclusive, again low doses of deltamethrin 0.7 and 1.2mg/kg and bioallethrin 0.72 and 72mg/kg were used. This time the mice showed an increase in behavioural activity 4 months after dosing compared to control animals. The neurochemical work showed some small changes in receptor expression after treatment which led Eriksson to suggest these chemicals were having permanent effects in adults as a result of exposure when young.

Our concern was obvious and we were one of the groups he mentioned in his article that established contact with him and in fact we invited him to CTL to give a seminar and to explore his work with him. He seems a pleasant and easy to get on with scientist. Our main point for discussion has been at a technical level around, the dosing of such small mice, the consequence of removing them from their mother at an early age for dosing, the type of behavioural systems used and how he manages to get such low standard deviations on his data, particularly the receptor binding work where most laboratories have a much larger variability. Based on peer pressure he has recently published that the effect with bioallethrin does show a dose response relationship.

CTL's response was to talk to the Medical Research Council(MRC) Toxicology Unit at the University of Leicester, Lewis Smith is the director and they were very interested in the work and decided to set up some studies. We were very keen to see whether the work could be reproduced in an independent laboratory outside of Sweden! We are funding a two-year post-doctoral fellow to work with Dr David Ray at Leicester which started about a year ago. Initially it was critical to ensure that the methods we used were as close as possible to those used in Uppsala, and in this regard Per Eriksson has been most helpful, the student spending some time in his laboratory seeing how the animals were dosed, how the behavioural measurements were made and the method of statistical analysis. We made the decision to use DDT as our model chemical to check out the method before we moved onto paraquat.

The latest position is that David Ray has conducted 2 experiments with DDT, one at 0.5mg/kg the same dose used by Eriksson and no behavioural effects were seen. However, in a second study using 0.5, 1 and 5mg/kg DDT some small statistically significant reductions in behaviour were seen at 0.5mg/kg, none at 1mg/kg and a small statistically significant increase at 5mg/kg. Overall the findings are unclear at present and David Ray needs to do the neurochemical measurements. Parathion and dieldrin have been through the same protocol and no changes were seen. Paraquat using a different strain of mouse (NMRI) to that used by Eriksson (C57bl6) was also negative. David Ray is currently repeating the paraquat work in the C57bl6 mouse.

Regarding the pyrethroids, Eriksson has used deltamethrin and bioallethrin, not any of interest to Zeneca. Eriksson's work has come to the attention of the German regulatory authorities(BGVV) and they have asked the industry to comment and further to give them some help in putting the findings into context. In this regard, Diane Castle and I recently attended an ad hoc meeting of the European pyrethroid manufacturers and it was decided to put

together a technical paper recommending a way forward. I was asked to chair this technical sub-group and we prepared a paper recommending that Dr David Ray be funded to repeat the studies, as an independent scientist, using bioallethrin and deltamethrin. This was agreed by the producers and accepted by the BGVV. A protocol for the study design is currently being circulated around the Companies for approval and financial support.

So the current position is that we are trying to see if the small changes reported by Eriksson can be reproduced in another laboratory, presumably they will be in time and then we need to address the significance of the findings. At present we have taken the more pragmatic approach, if they cannot be repeated then the pressure will be off!

It has taken rather longer than I thought to tell you our current position but I hope you can see our current thinking. If the results are reproduced I guess we (CTL and AgChem business) will need to decide whether we examine our lead pyrethroids.

There is one other issue on pyrethroids which has been recently been raised. Two papers, one from Italy and the other from the US EPA laboratories have reported that these chemicals (deltamethrin and cypermethrin) are more acutely toxic to young rats than adult rats. The papers are enclosed for your information. It is well established with other chemicals such as OP's that young animals are more sensitive, as their ability to detoxify chemicals is not fully formed. The authors claim that the increase in toxicity with deltamethrin is more than typically seen with other chemicals. MLD deltamethrin: adults, 81mg/kg; 21-day old animals, 11mg/kg; and 11day old animals 5.1mg/kg!

I hope this helps, if you have any queries please get in touch, **the findings to date by David Ray are changing as each experiment is conducted so please treat the information as provisional.**

With kind regards,
Yours sincerely,

Dr E A Lock.

ZENECA Agro

ZENECA Agro
Islands Brygge 41
2300 København S

Telefon Redacted - EU PII
Fax 32 88 82 75
Giro 5 43 05 34

Zeneca Central Tocicology Laboratory
Att. Dr. Ted Lock
Alderly Park
Macclesfield
Cheshire SK10 4TJ
England

10 May 1995
KHV/dtm

Dear Ted,

Re.: Swedish neurotoxicology research involving pyrethroids

In March there was article in one of the biggest Swedish newspapers in which Dr. Per Eriksson, the Institute for Environment and Development Biology at Uppsala University stated that exposure to chemical substances including pyrethroids and OP's has an effect on the brain of unborn children (for your information enclosed please find an internal translation of the article). I assume that this was the Swedish research you were thinking of when you during your visit to Denmark in November last year mentioned that it was possible to find effects which you were unable to confirm in repeat studies.

We are somewhat concerned about the fact that this is now being discussed publicly in Sweden, and in particular because the Swedish National Chemicals Inspectorate (NCI) is the rapporteur for lambda-cyhalothrin for the EU review. At a toxicology training course at CTL both Dr. Bob Scott and Dr. Ashley Wickramaratne recommended me to take contact to you concerning this issue.

In order to understand the issue a little better I would appreciate if you could send me some more details of Dr. Per Eriksson's findings, including which pyrethroids he have used in his tests if possible. Also I would like to have some information on what Zeneca has done in this area and where (MRC I understand - please describe what MRC is).

My plan is to give the information to our commercial manager in Sweden, Mr. Mogens Erlingson (for your information he is among many other things responsible for all our direct contact to the NCI, and he was the one who picked up the above mentioned article).

The objective is not that Mogens actively will pass on this information to anyone, but it would enable him to give a limited immediate verbal response, if/when the issue is raised during some of his regular contacts with the NCI or other researches in general. If this is not enough Mogens will contact me and/or Maureen Smith RAD, Fernhurst in order to get a more formal input ie. on paper or arrangement of a meeting (Mogens will not actively propose this).

Furthermore, for your information the Danish Environmental Protection Agency (DEPA) has published a review report concerning neurotoxicology. Attached please find a copy of the content and a summary. I can easily send you a copy of the report if you are interested. Please let me know.

Kind regards,
ZENECA Agro

Karl Henrik Vestergaard

c.c. Maureen Smith, RAD, Fernhurst.
Mogens Erlingson (letter only).

PQ/ CORRESPONDENCE

10 MAY 1995

From:
Jon Heylings, CTL
Alderley Park (Ext Redacted - EU PII)

Date:
05-May-1995

Document Ref:

To:
Ted Lock

Copies to:
Bob Scott
John Ishmael

PARAQUAT PLASMA PROFILES IN RAT AND DOG

Ted,

If I had known the purpose of the recent meeting with Iain I would have taken the attached graphs along. I would like to add some facts to our old chestnut.

As you can see, at both a sub-lethal (LD 25) and supra-lethal dose (LD 50-90) we observe little difference betwen the plasma PQ profile in rat and dog. I agree that there may be important species differences relating to lung retention of paraquat etc and may be if MgSO4 is given after 4 hours it will only work in the rat. That remains to be seen. However, I still maintain that the current rat data differs somewhat from the 0-30h rat profile for an LD50 dose of PQCl2 published by Lewis in the BMJ in 1974 (see attached).

A further important factor when considering plasma PQ data across species is the influence of the emetic when we move from PQCl2 to the formulated product, Gramoxone. Dogs are very sensitive to the emetic, and of course, rats have no vomit reflex. Thus, when we compare Gramoxone in rat and dog there <u>are</u> major differences in plasma profile ie much more prolonged in rats when given at LD50 and a sharp early peak followed by rapid clearance at LD50 in dogs since unabsorbed PQ is vomited out.

The limited human data with a full profile does look very similar to the dog, but almost invariably includes plasma PQ measured <u>after</u> treatment by gastric lavage, Fuller's earth etc, following an early admission. By removing unabsorbed PQ, there is no sustained plasma PQ in the 12-48 hour region which occurs without treatment. The level of emetic is only a threshold dose in man at LD50 and hence has little, or no effect, on the plasma PQ profile.

Increasing the concentration of the emetic by 5-10 fold would induce rapid vomiting in man (like the dog) and blood paraquat profiles would be similar in shape to that observed with Gramoxone in dogs. If such a change was made, or other methods of preventing PQ absorption ie Magnoxone were introduced by Agrochemicals, there would probably be little need for an antidote.

Despite my feelings on this I will still try to present a balanced case at he impending review. The issue of emetic potency will be avoided also. After all, despite the wealth of toxicological studies with PQ in rats and dogs, we will only discover any potential benefits when formulations or treatments are examined in man.

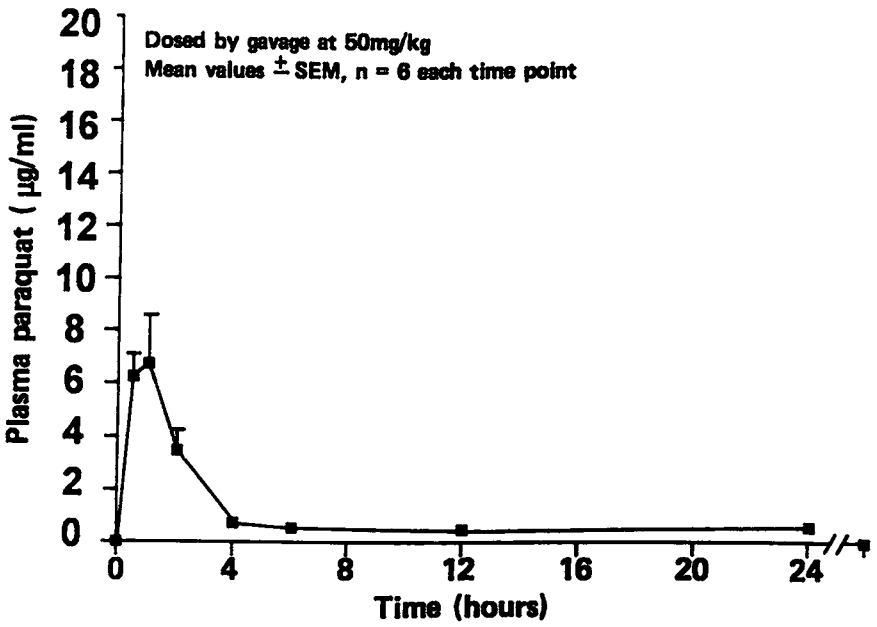
Nothing like a good bit of healthy scientific debate!

Regards,

Jon

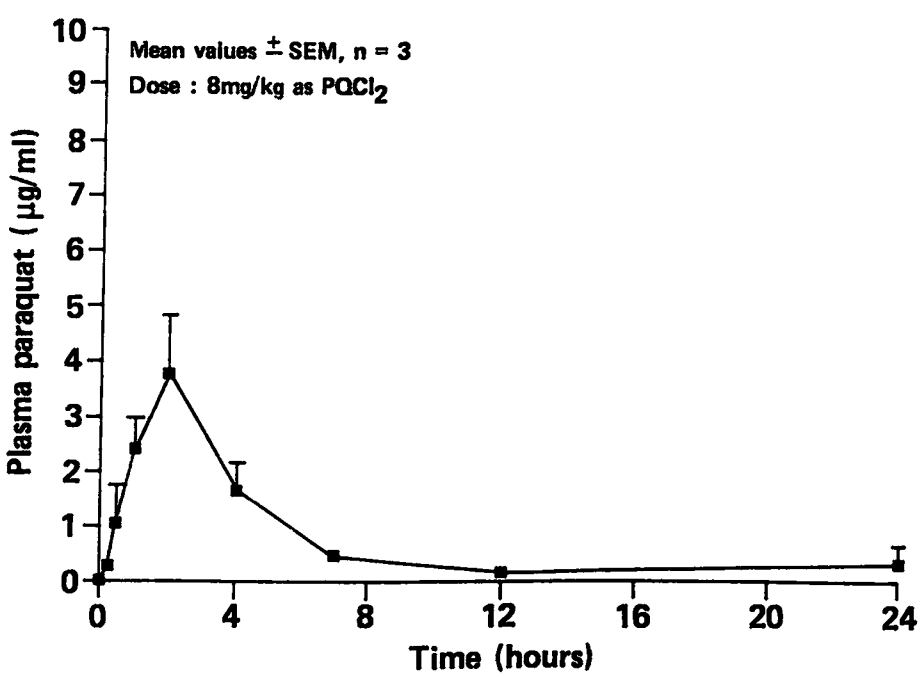
Rat LD 25

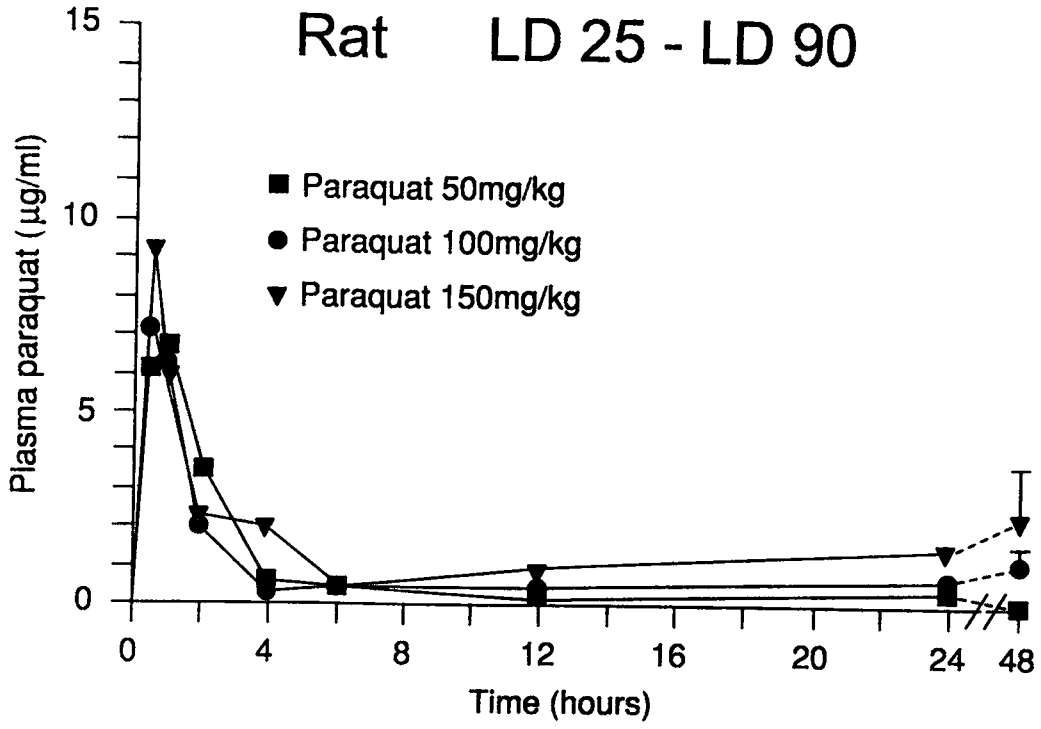
ABSORPTION OF PARAQUAT (DICHLORIDE) IN THE CONSCIOUS RAT 'IN VIVO'

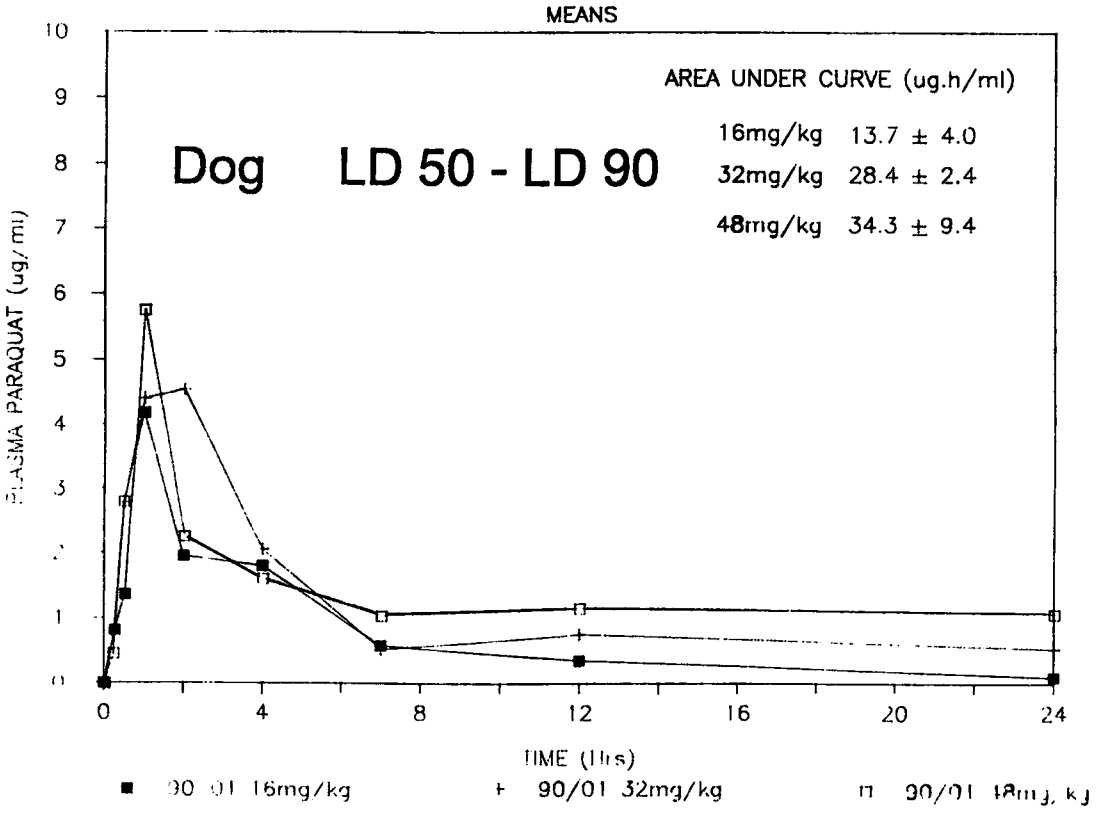


Dog LD 25

ABSORPTION OF PARAQUAT ($PQCl_2$) IN THE DOG




Rat LD 25 - LD 90
Paraquat 50mg/kg
Paraquat 100mg/kg
Paraquat 150mg/kg
Plasma paraquat (µg/ml)
15
10
5
0
0
4
8
12
16
20
24
48
Time (hours)


MEANS
AREA UNDER CURVE (ug.h/ml)
16mg/kg 13.7 ± 4.0
32mg/kg 28.4 ± 2.4
48mg/kg 34.3 ± 9.4
Dog LD 50 - LD 90
PLASMA PARAQUAT (ug/ml)
10
9
8
7
6
5
4
3
1
0
0
4
8
12
16
20
24
TIME (Hrs)
90/01 16mg/kg
90/01 32mg/kg
90/01 48mg/kg

development of the lung lesion, studies in experimental animals are necessary. The rat is probably the most extensively studied species in terms of the response of its lung to paraquat toxicity. In general, there are two distinct phases to the development of the pulmonary lesion. First, is a destructive phase in which the alveolar epithelium of the lung (type I and type II epithelial cells) are destroyed (Smith & Heath, 1974, 1976; Smith *et al.*, 1974a). If the destruction of the alveolar epithelium is extensive, an alveolitis develops, associated with pulmonary oedema and the infiltration of neutrophil polymorphs into the lung. In the case of rats, this acute alveolitis may be severe enough to cause death. The second phase of the lung lesion can be regarded as a consequence of the acute damage to the alveolar epithelium and resulting alveolitis (Smith & Heath, 1976). In this phase, an extensive intra-alveolar and interalveolar fibrosis develops which may be so widespread and severe as to destroy completely the normal alveolar architecture. With the obliteration of the alveolar membranes and their replacement with fibrous tissue, the opportunity for effective gaseous exchange is reduced and this may be severe enough to cause death from anoxia.

Uptake of paraquat from the gastrointestinal tract

When paraquat is administered orally to rats, the concentration of paraquat in the plasma is determined largely by the amount of paraquat present in the small intestine (Smith *et al.*, 1974b). Also, when dogs were given paraquat orally and drugs were administered to decrease stomach emptying time, the concentration of paraquat in the blood was decreased (Bennett *et al.*,1976). However, when dogs were given paraquat and drugs to increase stomach emptying time, the plasma paraquat concentration was increased compared with control animals (Bennett *et al.*, 1976).

These data from both rats and dogs indicate that the absorption of paraquat largely occurs from the gastrointestinal tract somewhere beyond the stomach. It is assumed that this is the case in humans, although there does not appear to be any good evidence as to the site of paraquat's absorption.

It was found after the oral administration of paraquat to rats that the plasma paraquat concentration remained relatively constant for 30 h, during which time the concentration in the lung rose progressively to several times that in the plasma (Figure 1). In no other organ studied was this time-dependent accumulation of paraquat detected (Rose *et al.*, 1976). However, it should be noted that the kidney, which is the organ responsible for the excretion of paraquat from the plasma, also has high concentrations in comparison with other organs. Thus the selective accumulation of paraquat into the lung and its high concentrations in the kidney provides a plausible explanation why these organs are those selectively damaged by paraquat following oral dosing (Smith *et al.* 1974b; Rose *et al.*, 1976).

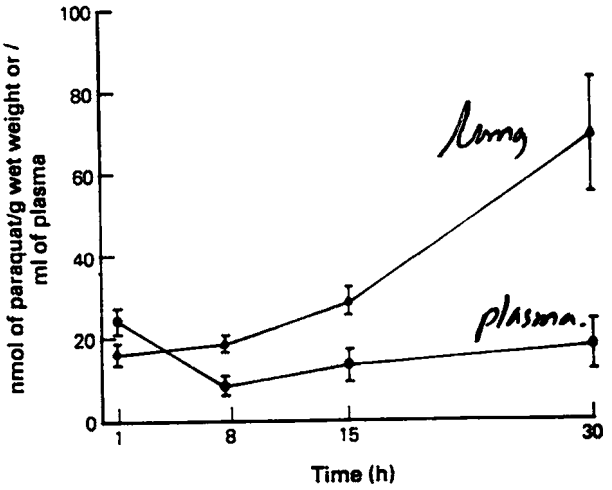


Figure 1 Relation between lung (▲) and plasma (●) concentrations of paraquat following an oral dose of paraquat. Level of paraquat in the lung and plasma of rats given 680 μmol of paraquat/kg body weight orally. Points on the graph represent mean ± SEM. At least five rats per time point [L. L. Smith, A. F. Wright, I. Wyatt & M. S. Rose (1974b) *Br. Med. J.*, iv, 569–571]

126 mg/kg 20 nmol = 3.7 μg

Accumulation of paraquat into the lung

Using lung slices, Rose *et al.* (1974) first described the time-dependent accumulation of paraquat into lung tissue. This process was found to be energy-dependent in so far as it could be inhibited by the addition of the metabolic inhibitors cyanide and iodoacetate to the incubation medium (Rose *et al.*, 1974). The accumulation of paraquat into the rat lung obeys saturation kinetics and various species of animal have the ability to accumulate paraquat (Rose *et al.*, 1976). As can be seen in Table 1, the apparent kinetic constants for the rat and human lung are similar, suggesting that the

Table 1 Apparent kinetic constants for the accumulation of paraquat into the lung of several animal species

Species	K_m (μM)	V_{max} ($nmol\,h^{-1}\,g^{-1}$)
Rat	70	300
Mouse	68	556
Syrian hamster	77	452
Guinea pig	96	49
Rabbit	0.05	20
Man	40	300

PQ / Correspondence.

- 3 MAY 1995

ZENECA

ZENECA Agrochemicals
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UK

Telephone (01428) 644061
Telex 858270 ZENAGR G
Telegraphic ZENAGCHEM
Fernhurst
Fax (01428) 652922

Mr F M Cunneen
ZENECA IRELAND LTD
PO Box 245A
College Park House
20 Nassau Street
Dublin 2

Via Fax : 6795864

Your Ref	Our Ref	Direct Line	Date
	GAW/BJS/L313	Redacted - EU PII	01 May 95

EU LOBBYING & PQ SUICIDES

Thank you for your 25 April note. Unless you have a specific reason for Philip Chambers to visit Fernhurst, it would seem to me more sensible that members of RAD should meet him during his proposed visit to CTL. May I propose that you arrange this directly with Dr John Doe and Dr Bob Scott, involving John Street, Andy Cook and myself?

I appreciate your forebearance over the fact that I have not yet been able to get back to you on the Paraquat and Suicide document and will do my best to put that right in the next few days.

With kind regards,

Yours sincerely

Barbara Sandford.

pp G A Willis
Manager
Regulatory Affairs Department

cc Dr J E Doe
Dr R C Scott
Mr J R Street
Mr A R Cook

FROM:
F M CUNNEEN

TO:
G A WILLIS
RAD/BSD
Zeneca Agrochemicals
Fernhurst

ZENECA IRELAND LTD
PO BOX 245A
COLLEGE PARK HOUSE
20 NASSAU STREET
DUBLIN 2

TEL: Redacted - EU PII
FAX: 6795864

OUR REF: FMC/PE

DATE: 25 April 1995

EU LOBBYING

When you were last in Dublin, I mentioned to you meeting Philip Chambers of Trinity College, Department of Toxicology. Regrettably, he was away for our Poisons Centre function and couldn't (despite having accepted our invitation), in the event, attend. However, he would be interested in visiting CTL and I wonder whether you would like to meet him there as he is very influential in the whole area of toxicology.

Conversely, we could arrange a visit to CTL and thence to Fernhurst. He is involved in the Scientific Advisory Committee of the European Community(C.S.T.E.) and has just got a three year extension to this. This will mean more to you than it does to me, but from general discussions I know he is well connected in the whole area of toxicology in Europe and, indeed, beyond.

On a separate topic, I look forward to your comments on the rough draft on *Paraquat and Suicide*. The plan is that we would issue a final version of this to a very small selected audience which would, hopefully, if the final version looks credible, persuade the audience concerned, e.g. Regulators, Poison Centre Managers, etc. that we have seriously looked at the issue of paraquat as a herbicide and haven't simply dismissed the issue of suicide as being purely a social problem over which we have no control and, in effect, none of our business. Hopefully, the final paper will reach this conclusion, but through the mechanism of a fairly argued paper.

Regards.

FACSIMILE MESSAGE

24 JUL 1995

From:
Dr B M Elliott
Regulatory Genetic Toxicology
Research Toxicology Section

ZENECA Central Toxicology Laboratory

Alderley Park Macclesfield
Cheshire SK10 4TJ England

Tel: Redacted - EU PII
Telex: 669095/669388
Fax: 01625 590249
EDT:

To:
A Cook
RAD
Fernhurst

Copies to:
Registry
R C Scott

Your Ref	Our Ref	Tel Ext	Date
	BME/VMC/003	Redacted - EU PII	21 Jul 1995

PARAQUAT : BACTERIAL ASSAY FOR GENE MUTATION

With reference to your letter of 11 July regarding the UK request for a bacterial assay for gene mutation for paraquat and your question as to whether existing studies can suffice.

Regarding the three reports CTL/C/1868, CTL/C/364 and CTL/P/243; all three were reviewed individually against the EU requirements as in the EEC method B14 and all have significant deficiencies (eg. positive controls not working or spontaneous levels being outside acceptable limits). This is the reason that only limited sheets were issued in the EC Review process, sufficient to indicate that the studies were inadequate by the guidelines.

I have looked at my notes made of the literature studies and for all of these there are again significant deficiencies against B14. These include lack of data on test sample source/purity and lack of experimental data. I do not believe therefore that there is a simple combination of studies that will satisfy the EEC B14 methodology.

However, in the case of paraquat, and for this request from the UK PSD, I would question the need for such a data-set. The value of the Ames test is in an assessment for the genotoxicity of the material and to contribute to the evaluation of possible carcinogenicity/mutagenicity. If we consider the data available for paraquat, the required data are already available and the conduct of an Ames test, whether the result is positive or negative, will make no difference to the interpretation of the database and lead to no additional actions. This last statement is made based on a scientific perspective and also on my understanding of the test strategy that the EU are operating. Thus, with regard to the genotoxicity issue, we can ask the question "Is paraquat genotoxic in vitro?". The answer is

yes, since paraquat is clastogenic in vitro. This result is consistent with the vast body of data available (much published) on the biochemical mode of action of the chemical (it forms oxygen free radicals). Since it is clastogenic in vitro, further studies have been conducted in vivo and paraquat is non-clastogenic in vivo. Furthermore, paraquat is negative in the in vivo rat liver DNA repair (UDS) study in vivo (as well as in other in vivo studies such as the dominant lethal assay). From a scientific perspective therefore, paraquat has been shown to be genotoxic in vitro, but in a range of in vivo studies has been shown to be non genotoxic in the whole animal. It actually does not matter whether paraquat was positive or negative in the Ames test, the above conclusion stands. My understanding of the latest EU strategy for genotoxicity evaluation of agrochemicals is that if the chemical is clastogenic in vitro then a cytogenetic study in vivo should be done. If the chemical is shown to be Ames positive (ie. cause gene mutations), then a liver DNA repair or mouse spot test in vivo should be done. We have, of course, done both of these already, hence my feeling that nothing more would be required regardless of the Ames study outcome.

Two other items are worth adding. Firstly, although we do not have an EEC B14 compliant study, paraquat has been evaluated in the Ames test by a number of laboratories and reported in the literature or company reports. The majority indicate an Ames negative profile. This is summarised in my genotox overview. Secondly, the biochemical mechanism of action of paraquat is very well understood and it is possible to make a scientific, knowledgeable assessment of its activity in certain systems. For example, paraquat can produce oxygen free radicals in the appropriate environment and such free radicals can damage DNA and cause toxicity. Hence, if conditions in vitro allow an excess of free radicals, then DNA damage/toxicity and positive results ensue (eg. clastogenicity). This has been identified biochemically, and also has been shown in the in vitro cytogenetic studies. The data are therefore available to move forward from the in vitro situation to the in vivo situation, as has happened.

I would have thought that the UK PSD would have been amenable to the above arguments/logic which indicate that apart from a pure box-ticking exercise, there is nothing to be gained from conducting an Ames test on paraquat. The above arguments should allow PSD to both proceed with the assessment of paraquat without a new Ames test and also justify their position to other EU authorities.

Please call me if you would like further discussion of this area or a To Whom It May Concern Letter. I would have thought that the EU process was mature enough to recognise the toxicology position of paraquat and not mandate an Ames test.

Regards

Barry Elliott

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