The recovery of plasma cholinesterase and erythrocyte acetylcholinesterase activity in workers after over-exposure to dichlorvos

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Plasma cholinesterase and erythrocyte acetylcholinesterase activities have a long history of use in monitoring both workers at risk of organophosphorus pesticide (OP) exposure and in investigating accidental exposures to OPs. On account of wide inter-individual variation, the establishment of unexposed, baseline enzyme activities is necessary for accurate interpretation. This paper describes the rate of recovery of the two enzymes' activity after substantial over-exposure of eight subjects to the OP dichlorvos. Plasma cholinesterase activity, immediately after exposure, was substantially more inhibited than erythrocyte acetylcholinesterase activity. The plasma enzyme activity showed an exponential pattern of recovery with a half-life of around 12 days, so recovery was essentially complete after about 50 days. This reported half-life of recovery is consistent with the reported de novo synthesis rate of plasma cholinesterase. The mean recovery of erythrocyte acetylcholinesterase activity appeared linear over time, attaining unexposed activity after about 82 days, which is somewhat shorter than the life-span of erythrocytes. These indicate the sort of time period, after an OP incident, before a valid unexposed level can be established in an individual; and substantiate the guidance given in the Health & Safety Executive's document MS17 on a minimum period of 60 days without exposure in order to establish pre-exposure baseline levels.

Key words: Acetylcholinesterase; biological effect monitoring; butyrylcholinesterase; DDVP; organophosphorus pesticide; pseudocholinesterase.
inhibition: spontaneous reactivation of enzyme activity, a blood sample needs to be taken to exposure in order that from any further potential OP exposure until enzyme activities return to normal. The rate of recovery of enzyme activity after exposure also defines how close a blood sample needs to be taken to exposure in order that an enzyme decrease is still detectable. Thus the time taken for recovery of pChE or AChE after interaction with OPs is an important factor.

The general structure of an OP is \( R_1 R_2 P(O)X \) or \( R_1 R_2 P(S)X \). The latter structure needs conversion to the former by enzymic oxidative desulfuration in tissues in order to be inhibitory to cholinesterases. The \( R_1 R_2 \) moiety of registered OPs are commonly dimethoxy (\( CH_3O- \)) or diethoxy (\( C_2H_5O- \)) groups. After interaction with an OP both pChE and AChE have their active site phosphorylated by the \( R_1 R_2 P(O)- \) group and are unable to carry out their normal enzymic function. Two reactions can occur subsequent to this inhibition: spontaneous reactivation of enzyme activity, where the phosphoryl group is hydrolysed from the active site; or ageing, where molecular rearrangement of the phosphoryl moiety gives an inactive enzyme where activity only returns by resynthesis of new enzyme. Clinical treatment of OP poisoning by oxime therapy can increase the rate of the former reaction, or spontaneous reactivation, but has no effect on aged enzyme.

This paper describes the return of pChE and AChE activity in a small group of workers who were overexposed to the OP, dichlorvos, during a period of several days and were removed subsequently from exposure until their enzyme activities returned to normal. These workers were under a health surveillance programme involving regular blood cholinesterase monitoring.

Dichlorvos (\( O, O \)-dimethyl-\( O-2,2 \)-dichlorovinyl phosphate, \( (CH_3O)_2 P(O)OCHCCl_2 \)), also known as DDVP, is a direct inhibitor of cholinesterases and represents one of the ‘dimethoxy OPs’ in contrast to the other common category of ‘diethoxy OPs’. Dichlorvos is different to other OPs in that it has a significant vapour pressure. This latter property has made dichlorvos a useful insecticide, being widely found in vaporizers used indoors to control flying insects. The noted significant vapour pressure of dichlorvos makes inhalation, as well as dermal contact, a common route of over-exposure. Currently available guidance suggests that the route of exposure to dichlorvos indicates the type of symptoms presenting after over-exposure. Dichlorvos is rapidly eliminated from the body after metabolism by enzymes found in tissues and blood. The metabolism of this volatile and directly inhibitory OP means that symptoms of exposure are noted for the rapidity of both onset and recovery in comparison to other OPs.5

RESULTS AND DISCUSSION

Figure 1 shows the results of pChE and AChE activity in the 20 subjects monitored around the time the problem with the dichlorvos impregnation system was identified. The data confirmed that over-exposure to dichlorvos was occurring. The monitoring of pChE and AChE in the eight workers was carried out a number of times at intervals, after taking the index blood sample, until enzyme activity recovered. No therapy was used during the recovery period, including oxime treatment, which may accelerate spontaneous reactivation of unaged cholinesterase enzyme. Therefore the reported results reflect the normal return of enzyme activity due to any spontaneous reactivation of inhibited enzyme and de novo synthesis of enzyme.

POPULATION AND METHOD

Subjects in this study were involved with production equipment, impregnating absorbent pads with dichlorvos and their subsequent incorporation into vaporization units. At about the time of the index blood samples the production equipment was causing problems, which frequently meant greater potential dermal exposure to the workers. Shift patterns had also concurrently changed, which meant that some subjects were working longer, consecutive daily shifts on this production line. Mean measured atmospheric dichlorvos levels at this time were 1.15 mg/m\(^3\), the current occupational exposure limit being 0.92 mg/m\(^3\)³.7

During work subjects had begun reporting relatively minor symptoms of a flu-like nature, tiredness and respiratory symptoms of wheezing and tightness of the chest. The respiratory symptoms were most prominent, leading to local concerns as to whether dichlorvos could cause asthma or an allergic response.8,9 It was recommended that blood samples, for cholinesterase measurements, should be taken from subjects complaining of symptoms and from all those who had recently worked, or were working shifts, on the dichlorvos pad impregnation line.

Blood samples from 20 subjects were measured for cholinesterase activity around this time. Blood cholinesterases measurements (pChE and AChE) were measured by a long established method used for routine monitoring. The automated method uses acetylthiocholine as substrate and is performed at 37°C. Samples are analysed on the day of receipt in the laboratory and any results indicating a significant depression in enzyme activity are reported immediately to the originating occupational health professional. Index samples from eight of the 20 subjects were likely to have been taken, at the most, 1 day after the end of their exposure and they had lower AChE activities. These eight subjects form the basis of the data for this paper. pChE and AChE activities in their index samples were between 3 and 15% and 24–43% of their respective baseline activities (100%). These, together with several other workers, were removed from any possible OP exposure immediately, once results of the index sample were known. Monitoring of pChE and AChE in the eight workers was carried out a number of times at intervals, after taking the index blood sample, until enzyme activity recovered. No therapy was used during the recovery period, including oxime treatment, which may accelerate spontaneous reactivation of unaged cholinesterase enzyme. Therefore the reported results reflect the normal return of enzyme activity due to any spontaneous reactivation of inhibited enzyme and de novo synthesis of enzyme.

RESULTS AND DISCUSSION

Figure 1 shows the results of pChE and AChE activity in the 20 subjects monitored around the time the problem with the dichlorvos impregnation system was identified. The data confirmed that over-exposure to dichlorvos was occurring with the then current work practices and immediate remedial action was undertaken. A number of workers were removed from any further OP exposure and the production line was halted for relocation and improvement.

It is noted that the depressions in pChE and AChE activity are substantial. Nineteen of the 20 pChE measurements were less than the 70% pChE of baseline
levels noted in HSE MS17, as triggering medical examination and possible suspension from further exposure. Thirteen of the 20 subjects had AChE levels which were less than the 70% of baseline levels recommended currently by OSHA, for removal from further exposure. The eight subjects monitored longitudinally in this paper had the lowest AChE levels (24 – 43% of their respective baseline activities). The relationship between depression in AChE activity and toxic symptoms has been noted as being complex, depending on the OP structure, the route of exposure and the nature of exposure (single acute versus repeated). Multiple exposures causing a more gradual reduction in cholinesterase activity are tolerated better than a single acute exposure. It is likely in this occupational study that the workers had suffered higher than usual dichlorvos exposures over a number of shifts during problems with the production equipment. This may have led to the subjects tolerating large reductions in blood AChE without obvious evidence of substantial systemic toxicity. It is interesting to note that respiratory symptoms appeared to predominate for this OP, which has a substantial vapour pressure, although average measured atmospheric levels were only marginally over the occupational exposure standard. This may suggest that dermal exposure substantially contributed to the cholinesterase inhibition.

Table 1 shows the measured pChe and AChE activities in the eight subjects during recovery. pChe for the cohort of eight subjects showed an exponential recovery of activity (Figure 2) which, when fitted to a single phase exponential association curve, gave a curve fit of $r^2 = 0.96$ and a half-life of recovery of 11.8 days (95% confidence interval = 10.4 – 13.5 days). In contrast, the recovery of AChE showed a linear profile (Figure 3), which when fitted by linear regression gave a fit of $r^2 = 0.845$ with a y axis intercept of 33.4% (confidence interval = 28.4 – 38.4%). The recovery of AChE activity was 0.807% of baseline activity per day.

These two patterns of enzyme recovery reflect the type of enzyme removal from circulation and de novo synthesis. The pChe half-life appears to be governed by stochastic abstraction by the liver, of a percentage of the circulating pChe, without apparent regard to the age of pChe or whether the pChe was OP-inhibited or not. AChE recovery appears largely determined by the
replacement of senescent erythrocytes of around 110 days old by new erythrocytes. In terms of an OP exposure where erythrocytes of all ages may have their AChE equally inhibited, this gives a linear as opposed to an exponential graph for replacement of inhibited AChE enzyme. The AChE activity appears to recover to 100% in around 82 days (confidence interval = 72 – 98 days), which is somewhat shorter than the life-time of the average erythrocyte. This apparent faster recovery may reflect that spontaneous reactivation of OP-inhibited AChE also has some part to play in the return to normal activity, or possibly that inhibition of acetylcholinesterase on the erythrocyte membrane confers some shortening of the normal life-span of that cell.

The half-life of pChE recovery of approximately 12 days after dichlorvos inhibition as found in this study is within the range of reports for the half-life of pChE (3 – 16 days). This reported range of values for pChE half-life was obtained from a number of different techniques including plasmapheresis, cholinesterase therapy in individuals with the silent cholinesterase gene and after administration of the radiolabelled organophosphate, diisopropylfluorophosphate. The agreement in half-lives between this study of dichlorvos exposed subjects and those studies using non-OP techniques suggests that the pChE was fully aged at the time of the index blood sample. This is in agreement with the rapid rate of ageing of pChE for dimethoxy OPs, such as dichlorvos, as previously reported in an in vitro study from this laboratory. Dimethoxy OPs, as a class, have the same rate of ageing and spontaneous reactivation from the same inhibited enzyme. Therefore these data are applicable to all dimethoxy OPs, with the caveat that high doses of the more lipophilic OPs may be stored to some extent in body fat from where they leach out slowly, possibly extending the apparent rates of pChE and AChE recovery.

As in this incident, our experience indicates that pChE activity invariably shows relatively greater depression than AChE from baseline values in samples taken soon after sub-acute or multiple exposure to OPs. The data suggest that the degree of pChE inhibition immediately after exposure caused by dimethoxy OPs will be reduced by 50% after a post-exposure period of around about 12 days. For AChE, the reduction of the initial (lesser) inhibition will be around 15% ((12/82) x 100), 12 days after exposure. If significant inhibition of AChE has been caused then it will be apparent for a longer period than for pChE. For diethoxy OPs, where the rate of spontaneous reactivation and ageing may be slower than for dimethoxy OPs, the pattern of pChE and AChE recovery may be different. However, if a substantial amount of the inhibited enzyme is in the aged form the recovery is likely to be similar to that seen in this report.

After a possible incidental dimethoxy OP exposure, the data would suggest that a minimum of a 30 – 40 day interval is pragmatically necessary before taking a subsequent blood sample for comparison. This subsequent sample would establish a ‘baseline’ measurement at around 80 – 90% of true baseline for pChE, but could still be far from a true baseline value for AChE if this enzyme had showed any inhibition. It would theoretically need an interval of 3 months between exposure and baseline measurement for both enzymes to have returned to pre-exposure levels. This analysis of the data shows the problems and inadequacy of pChE and AChE in investigating subclinical, accidental OP exposures.

These data may be useful to those health professionals who need to interpret blood cholinesterase results from individuals exposed to OPs. This includes health surveillance programmes which include establishment of baseline pChE and AChE values after a period of no OP exposure and the interpretation of results from reported potential OP exposure incidents.

REFERENCES


