N, N'-Dichlorobis (2,4,6-trichlorophenyl) urea (CC-2) is a potent sulphur mustard (SM) decontaminant developed by Defence Research & Development Establishment Gwalior, India. Formulations based on CC-2 have exhibited excellent protection in laboratory animals. The encouraging results have prompted further impetus for its development as a potential SM decontaminant in event of a chemical war. However, there is a paucity of information on preclinical pharmacokinetics of CC-2. Pharmacokinetic data is not only important to determine ADME of the molecule under development but is also a mandatory requirement by various regulatory agencies. In the present work an attempt has been made to generate some relevant preclinical pharmacokinetic data of CC-2.

A sensitive, precise and accurate bio-analytical method is a prerequisite to generate reliable pharmacokinetic data for an agent. Therefore, HPLC methods for quantitative determination of CC-2 in different bio-matrices were developed and validated. The parameters considered for validation included specificity, sensitivity, accuracy and precision and serum stability on storage and after three freeze-thaw cycles. All parameters were within acceptable limits proposed by regulatory bodies.

Pharmacokinetic behaviour of CC-2 following dermal application (200 mg/ml) and oral administration (0.5, 1.0 and 2.0 g/kg) was studied in male Sprague-Dawley rats. After dermal or oral administration, CC-2 was readily but not appreciably absorbed. Irregular concentration time profile following oral administration was observed which is probably due to its high lipophilicity and poor aqueous solubility. At the studied oral doses, CC-2 exhibited non-linear pharmacokinetics. Elimination half-life ($t_{1/2}$) following dermal route was higher than oral route, which is typical for dermal route of application. Absolute bioavailability of CC-2 following dermal application could not be determined due to its insoluble nature in commonly used solvents for preparation of i.v. formulation. Mean relative bioavailability following topical application in comparison to oral route was 43.2%. No visible adverse effects were observed in rats during studies.

*In vitro* protein binding of CC-2 was determined by charcoal adsorption method due to non-specific binding of CC-2 to ultrafiltration devices. At 1 μg/ml concentration (n=3), protein binding of CC-2 was found to be 70%. Excretion studies at 1 g/kg, p.o. dose indicated that CC-2 was not excreted in urine samples and only
1% of the administered drug was excreted unchanged in feces during 0-72 h. The results suggest the possibility that CC-2 might be getting extensively metabolized.

The present work indicated that topical application of CC-2 would result in low systemic exposure. However, characterization of distribution and elimination properties of CC-2 needs further work.

(PART-B)

Centchroman [INN: Ormeloxifene (CC)] is a non-steroidal once a week oral contraceptive developed by Central Drug Research Institute, Lucknow. It is a unique need-oriented contraceptive being effective when taken routinely as a weekly pill. The contraceptive action of CC has been well established in rodents, primates and humans and its anti-fertility action is quickly reversible. It has been included in the National Family Welfare Programme of Ministry of Health and Family Welfare, Government of India in the year 1995.

Today, CC is being used by healthy women of reproductive age as an oral contraceptive and is likely to be used in future for the treatment of breast cancer and/or osteoporosis. Therefore, concurrent administration of other drugs in various clinical situations is imperative. Such situations have great potential for drug-drug interactions. Interactions of certain therapeutic agents with hormonal contraceptives have been reported in clinical situations. Interactions resulting in efficacy failure of CC on tetracycline co-administration in laboratory animals have been reported. This prompted to conduct pharmacokinetic and pharmacological interaction studies of CC with certain classes of therapeutic agents with an aim to generate a preclinical database for future reference. The aim of the present work was to investigate interaction potential of concurrently co-administered antibiotics, antihypertensives, antiasthamatics and antidiabetics on pharmacokinetic and pharmacological profile of CC.

A reported HPLC bioanalytical method for simultaneous quantitation of CC and 7-DMC was reproduced with minor modifications and validated in rat serum prior to its application for sample analysis. The validated method was used to generate pharmacokinetic profile of CC with and without co-administered drugs. The validation parameters were found to be within acceptable limits.

The pharmacological screening method for assay of contraceptive efficacy of CC has been well established and validated in rats. The same method was employed
to generate pharmacological interaction data of CC in mated female rats after post coital oral administration of CC with above mentioned class of drugs. In case(s) where pharmacokinetic and pharmacological findings were not complementing each other, estrogenic and antiesterogenic activities of CC with the co-administered drug were evaluated in immature ovariectomized rats.

Baseline pharmacokinetic data of CC was generated at its contraceptive dose (1.5 mg/kg, p.o.) prior to pharmacokinetic interaction studies and was used as control. CC exhibited irregular serum concentration time profile with two concentration maxima ($C_{max 1}$: 59.5 ± 2.1 ng/ml, and $C_{max 2}$: 48.0 ± 2.3 ng/ml) at 1.5 and 6 h, respectively. The double peak phenomenon of CC is attributed to its discontinuous absorption across the GIT. Elimination $t_{1/2}$ (31 h), $Cl$ (0.73 L/h/kg) and $V_d$ (32 L/kg) were similar to earlier observations. Absolute bioavailability ($F$) of CC was found to be 0.72. Serum concentration-time profile of 7-DMC was irregular and variable and could be quantified up to 72 h post CC dose. The $AUC_{0-24}$ was found to be 474 ng.h/ml. The results of the study were in agreement with earlier reports.

In pharmacokinetic interaction studies with antibiotics (ciprofloxacin, cefixime, amoxicillin and metronidazole), CC showed double peak phenomenon except for amoxicillin which exhibited a single $C_{max}$ occurring at 4 h. The rate and extent of absorption of CC was altered in most of the cases. The major affect was observed on $AUC_{0-24}$ which was significantly lower than that in control. Other pharmacokinetic parameters were not affected significantly. The levels of 7-DMC were irregular and variable.

Studies with antihypertensive agents (amlodipine and atenolol) showed that amlodipine had pronounced affect on $t_{max 2}$ of CC. The $AUC_{0-24}$ and $AUC_{0-last}$ were significantly lowered by both amlodipine and atenolol. The $Cl$, $V_d$ and elimination $t_{1/2}$ of CC in amlodipine co-administered group were comparable to control data. With atenolol co-administration, the bioavailability of CC decreased to 0.47 than the control group. In both studies, 7-DMC could be observed up to 72 h with levels similar to control.

The pharmacokinetic profile of CC with co-administration of antiasthamatic agent theophylline resulted in similar $t_{max}$ but significantly lower $C_{max}$ in comparison to the control data. The $AUC_{0-24}$ and $AUC_{0-last}$ showed significant decrease than that observed in control group. Other pharmacokinetic parameters such as elimination $t_{1/2}$
and $V_d$ decreased but $Cl$ remained unchanged. 7-DMC started appearing at 1.5 h post theophylline co-administration and could be quantitated up to 48 h with $AUC_{0-\text{last}}$ being 307 ng.h/ml.

The antidiabetic agents (metformin, pioglitazone and glibenclamide) had similar affect on pharmacokinetics of CC on co-administration. The $AUC_{0-24\ h}$ and $AUC_{0-\text{last}}$ were significantly lower in comparison to control in all cases. Other pharmacokinetic parameters such as elimination $t_{1/2}$, and $Cl$ and $V_d$ were unchanged with respect to control except for pioglitazone where elimination $t_{1/2}$ and $V_d$ were lower than control. Following pioglitazone or glibenclamide co-administration, 7-DMC was detected up to 72 h whereas with metformin, it could be quantified up to 24 h. The profile of 7-DMC was however irregular in all cases.

In pharmacological interaction studies, no interaction (efficacy failure) of CC was observed on co-administration of either of ciprofloxacin, cefixime, metronidazole, amlodipine, atenolol, theophylline, metformin, pioglitazone or glibenclamide. However, amoxicillin co-administration interfered with contraceptive efficacy of CC in 66% of the rats. These findings were not correlating with the observed pharmacokinetic results. This may be due to the wide therapeutic index of CC or insignificant changes in formation of active metabolites. It is also possible that the changes in the serum levels of CC do not result in changes in the receptor milieu and hence the observed efficacy failure of CC can be purely pharmacodynamic in nature. To dwell further, the mode of interaction with amoxicillin, estrogenic and antiestrogenic activities of CC were tested in immature, ovariectomized rats. The results indicated that amoxicillin did not alter estrogenic and antiestrogenic activities of CC. Further studies are needed to establish the underlying phenomenon.