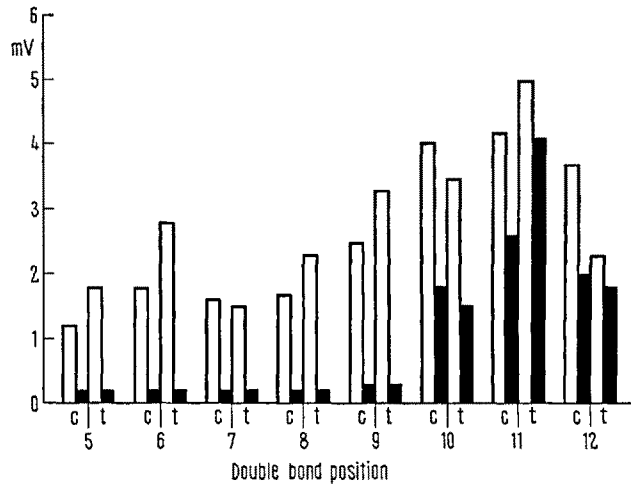
Table II. Field tests with different concentrations of the larch bud moth attractant, *trans*-11-tetradecenyl acetate, conducted in the Engadin (Switzerland)

| Attractant conc. in $\mu$l[a] | No. of males trapped August | | September |
|---|---|---|---|
| | 13–19 | 13–16 | 16–27 |
| 1 | 46 | – | – |
| $10^{-1}$ | 46 | 165[b] | 295[b] |
| $10^{-2}$ | 236 | 147[b] | 187[b] |
| $10^{-3}$ | 184 | 65[b] | 134[b]/100[c] |
| $10^{-4}$ | – | 71[d] | 90 |
| $10^{-6}$ | – | 160[d] | 213 |
| Control[e] | 125.5 | 174.5 | – |

Except for $10^{-3}/10^{-4}$ and $10^{-2}/10^{-6}$ $\mu$l, all differences between concentrations are significant at 2% level.
[a] 1 $\mu$l of attractant or acetone solution on a rubber stopper. [b] Same preparation as in August. [c] Mean of 4 replicates with fresh preparation. [d] Trap with fresh preparation. [e] Mean of the 4 best out of 10 traps containing virgin females.




Antennal responses of male larch bud moths to acetates (white columns) and alcohols (black columns) of 14-carbon chain standards with different position of double bond in *cis* (c) or *trans* (t) configuration (10 $\mu$g on filter paper, 4 replicates).

sponses. The acetate standards were more active than the corresponding alcohols, with *trans*-11-tetracedenyl acetate always producing the greatest response (Figure). The pattern of the responses obtained with the standards is similar to that of other species which use a monounsaturated acetate as a sex attractant[2,3,5]. The above data indicate that *trans*-11-tetradecenyl acetate may be similar to the main component of the sex pheromone communication system of this insect.

Field testing studies proved that *trans*-11-tetradecenyl acetate is very attractive to male larch bud moths but not for females. A test involving *cis*- and *trans*-11-tetradecenyl acetates and alcohol showed that only the *trans*-acetate is attractive, although 2 $\mu$l of chemical in a polyethylene cap did not begin to attract until it had been in the field for 40 days (living females did attract males during this period). Dilution series of the *trans*-acetate were then tested in an attempt to optimize the release rate (Table II). It became obvious that lower release rates were essential, that of 100 $\mu$g of chemical or more on a rubber stopper being too high. However, traps with 100 $\mu$g of the substance became highly attractive in the course of 1 month. The fact that, of the fresh preparations, 10 $\mu$g and 1 ng of chemical were most attractive, suggests that 2 optimum concentrations exist. The significance of this finding has to be studied during the next season. Since the fresh 1 ng trap contained only about 2 trillion molecules and since it did not lose its attraction for at least 14 days, one may conclude that a few molecules per $\mu$l of air suffice to attract the male larch bud moths.

Although it has not been rigorously proven that *trans*-11-tetradecenyl acetate is identical with the natural sex pheromone, the compound is potent enough in the field to replace living female monitoring traps and to be used in insect control experiments. This same compound has been found to serve as an attractant for several other lepidopterous species, but the most interesting point concerning its structure is that it represents the first case in which a species of the subfamily Olethreutinae apparently utilizes a 14-carbon rather than a 12-carbon chain attractant[5].

*Zusammenfassung.* Mit Hilfe der Antennogramm-Methode wurde *trans*-11-Tetracedenylacetat als spezifisches Attraktans für Männchen des Lärchenwicklers *Zeiraphera diniana* bestimmt. In geeigneter Konzentration ist die Attraktivität der synthetischen Substanz derjenigen virginer Weibchen ebenbürtig.

W. L. Roelofs and R. Cardé[6];
G. Benz and G. von Salis[7]

*Department of Entomology, Cornell University, New York State Agricultural Experiment Station, Geneva (New York 14456, USA), and Department of Entomology, Swiss Federal Institute of Technology Zurich, CH-8006 Zürich (Switzerland), 8 October 1971.*

[5] W. Roelofs and A. Comeau, Proc. 2nd Int. Congr. Pest. Chem., IUPAC (Tel Aviv 1971).
[6] This research was supported in part by Environmental Protection Agency Grant No. EP-00802 and by the Rockefeller Foundation.
[7] Contribution No. 46 of the research team for the investigation of the population dynamics of the larch bud moth, directed by Professor Dr. P. Bovey. The research was aided by the Swiss National Funds for Scientific Research.

## Distribution of the Herbicides 2,4,5-T and 2,4-D in Pregnant Mice. Accumulation in the Yolk Sac Epithelium

The teratogenic action of herbicides of phenoxyacetic acid type has been the object of several investigations. When 2,4,5-T was administered to pregnant mice, malformations such as cystic kidney and cleft palate were revealed[1,2]. In the first study[1] the impurity 2,3,7,8-tetrachlorodibenzo-*p*-dioxin[3] has been suggested to have contributed to the malformations observed, but later both purified 2,4,5-T and the dioxin were shown to cause

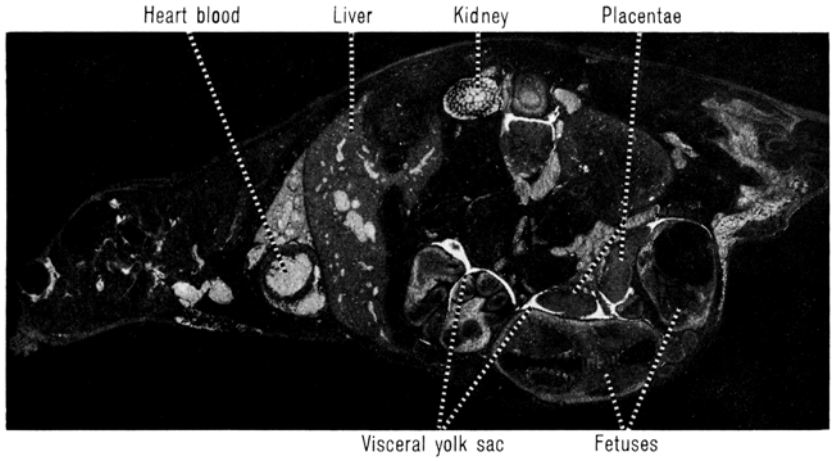



Fig. 1. Autoradiogram of a pregnant mouse 16 h after i.v. injection of $^{14}$C-2,4,5-T. The highest concentration is seen in the visceral yolk sac epithelium.

fetal damage[2]. It has also been suggested that there might be a synergistic effect between the two compounds with respect to their teratogenic action[4].

In our studies, pregnant mice were given i.v. injections of 1-$^{14}$C-labelled 2,4,5-T (The Radiochemical Centre, RCC, Amersham, England, specific activity 30 mC per mmole) or of 1-$^{14}$C-labelled 2,4-D (RCC, specific activity 34 mC per mmole). Both substances were dissolved in 50% ethyl alcohol and 10 μC of each substance was administered corresponding to 0.09 mg 2,4,5-T and 0.05 mg 2,4-D. The mice were of NMRI strain and weighed between 25 and 35 g. Whole body autoradiography was performed according to a method, described earlier[5].

Labelled 2,4,5-T was administered to 2 pregnant mice at early pregnancy. One mouse was injected 8 days after mating as determined by observation of vaginal plug and killed 4 h after injection. The other one was injected 9 days after mating and killed 24 h after injection. Labelled 2,4,5-T was also administered to a series of 8 mice at late state of pregnancy. The mice were sacrificed 5 min, 20 min, 1 h, 4 h, 16 h, 24 h, 48 h and 4 days after injection.

Labelled 2,4-D was administered to a series of 7 mice at late state of pregnancy. The mice were sacrificed 5 min, 20 min, 1 h, 4 h (2 animals) and 24 h (2 animals) after injection.

Mice at early state of pregnancy: After injection of $^{14}$C-2,4,5-T to the dam, the radioactive substance did not to any appreciable extent reach the embryo. The only organs with higher concentrations than the blood were the kidneys and the visceral yolk sac epithelium.

Mice at late state of pregnancy: Following administration of $^{14}$C-2,4,5-T the distribution within the dam was uniform, the only organs with higher concentrations than the blood being the kidneys and the visceral yolk sac epithelium. As early as 5 min after injection, the concentration in the yolk sac epithelium exceeded that in the blood. Later (after 20 min to 48 h) there was a further accumulation and retention in the yolk sac, while the concentrations in other tissues slowly decreased (Figure 1). The level in the serous fluids equalled that of the blood. The concentration in the brain was low. The choroid plexa showed activity similar to that of the blood.

[1] K. D. COURTNEY, D. W. GAYLOR, M. D. HOGAN, H. L. FALK, R. R. BATES and J. MITCHELL, Science *168*, 864 (1970).
[2] U.S. Congress 1970. Statement before the Subcommittee on Energy, Natural Resources and Environment of the Senate Commerce Committee, April 15, by K. D. COURTNEY et al.
[3] U.S. Congress, 1970, Statement before the Subcommittee on Energy, Natural Resources and Environment of the Senate Commerce Committee, April 15, by J. E. JOHNSON.
[4] U.S. Congress 1970, Statement before the Subcommittee on Energy, Natural Resources and Environment of the Senate Commerce Committee, April 15, 1970, by S. EPSTEIN.
[5] S. ULLBERG, Acta radiol., suppl. *118* (1954).

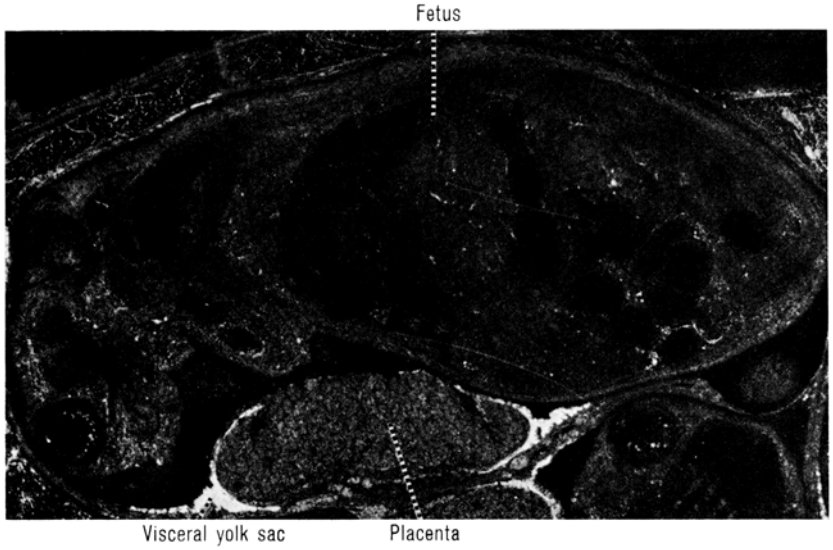



Fig. 2. Detail of an autoradiogram of a pregnant mouse 4 h after i.v. injection of $^{14}$C-2,4,5-T. Note accumulation in the visceral yolk sac epithelium. No accumulation is seen in the fetus.

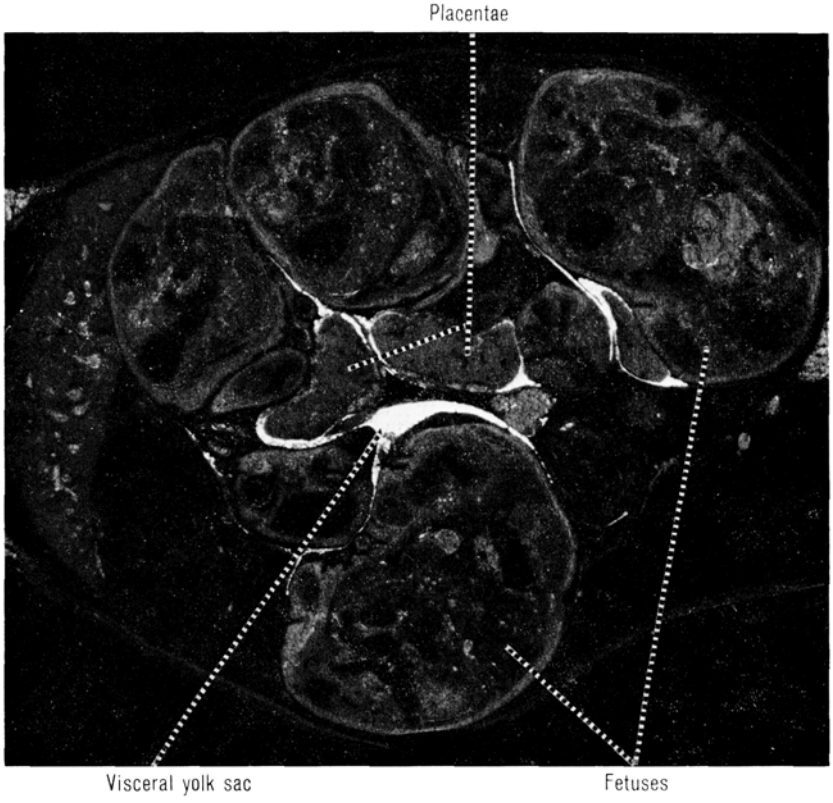



Fig. 3. Detail of an autoradiogram of a pregnant mouse 16 h after i.v. injection of $^{14}$C-2, 4, 5-T. Note accumulation in the visceral yolk sac epithelium. No site of accumulation in the fetal tissues is seen.

The activity slowly passed the placenta, and the fetal tissues gradually reached a similar level to the maternal ones. The highest activity was found in the fetal blood. There was no site of accumulation in the fetal tissues (Figures 2 and 3). Concentrations equal to that in the blood could be observed in serous fluids (the abdominal, pericardial and ocular liquids).

Labelled 2, 4-D showed a slight tendency for accumulation in the visceral yolk sac, passed to the fetus and was rapidly (within 24 h) eliminated from all tissues, including the visceral yolk sac. There was a great discrepancy in the disappearance rate of $^{14}$C-2, 4, 5-T and $^{14}$C-2, 4-D[6]. Both 2, 4, 5-T and 2, 4-D have proved to be stable in the body, no major metabolites being found[7]. It is therefore likely that the radioactivity in the autoradiograms represents only the unaltered substances.

*Discussion.* The evenness in distribution pattern which was observed both for 2, 4, 5-T and 2, 4-D is remarkable, with lack of accumulation in all tissues except the visceral yolk sac epithelium. In spite of the rather even distribution of both substances, the difference between them is striking with a much more rapid disappearance from all tissues for 2, 4-D and a stronger accumulation in the yolk sac epithelium for 2, 4, 5-T.

The selective uptake of 2, 4, 5-T in the yolk sac epithelium and the lack of placental transfer in the early pregnancy indicate a similar teratogenic mode of action for 2, 4, 5-T to the one which has been postulated for trypan blue[8]. This azo-dye was shown to be a potent teratogenic substance when administered to rodents[9]. Trypan blue was found not to pass the placental barrier, but to accumulate in the yolk sac epithelium[10]. LLOYD and BECK[8,11,12] have proposed that trypan blue acts by enzymatic inhibition of the embryotrophic nutrition. The early embryonic nutrition is largely carried out by enzymatic breakdown of nutritional macromolecules. Trypan blue has no teratogenic effect on rats after the 10th day of pregnancy, when the chorio-allantoic placenta assumes the function of nutrition of the fetus. Trypan blue is teratogenic, but not its reduction products *o*-tolidine and 2, 8-diamino-1-naphtol-3, 6-disulphonic acid.

The morphology of the placenta and related structures shows a great variation among species. In rodents, the yolk sac is the only extraembryonic tissue capable of breaking down macromolecules to an appreciable extent, but in other mammalian forms the embryotrophic nutrition is carried out by a wide variety of fetal membranes and disturbance of the embryotrophic nutrition migh be an important teratogenic mechanism[12]. It is, however, likely that the embryotrophic nutrition plays a quantitatively more dominant role for rodents than for primates.

*Zusammenfassung.* Nach Injektion des Herbizids 2, 4, 5-T wurde bei der Maus im Dottersackepithel eine selektive Kumulation beobachtet. Bei früher Trächtigkeit passiert die Substanz die Placenta nicht. Der Wirkungsmechanismus dürfte mit dem des Trypanblaus identisch sein.

N. G. LINDQUIST and S. ULLBERG

*Department of Toxicology,*
*University of Uppsala,*
*S–751 05 Uppsala (Sweden), 10 June 1971.*

[6] W. L. ZIELINSKI JR. and L. FISHBEIN, J. Agr. Food. Chem. *15*, 841 (1967).
[7] K. ERNE, Acta vet. scand, *7*, 264 (1966).
[8] J. B. LLOYD and F. BECK, Proc. of the International Symposium on Teratology, Como, 1967. Amsterdam: Excerpta Medica Foundation.
[9] J. GILLMAN, C. GILBERT, T. GILLMAN and J. SPENCE, S. Afr. J. med. Sci. *13*, 47 (1948).
[10] E. GOLDMANN, Beitr. klin. Chir. *64*, 192 (1909).
[11] J. B. LLOYD and F. BECK, Lab. Anim. *2*, 171 (1968).
[12] F. BECK and J. B. LLOYD, Lab. Anim. *2*, 157 (1968).