Effects of exposure to monocrotophos, an organophosphate on the ovary with particular refernce to general lipids and phospholipids in female albino rats

Dr. Kavita Arora

Department of Zoology, D.A.V. College, Abohar, Punjab, India

Abstract

The albino female rats were exposed to monocrotophos, an organophosphate at 1/5th of LD50 dose (14 mg/kg body weight) for 15 days (TI group), 30 days (TII group), 30 days with recovery period of 15 days (R group). Other three groups were kept as corresponding controls for all the treated groups and were fed on normal diet. At the end of the treatment, blood was collected for serum preparation to estimate triglycerides and phospholipids biochemically. For histochemical studies, paired ovaries in proestrous phase of estrus cycle were removed from various groups and the techniques for the fixation of lipids and the preparation of slides were used. During present studies, increase in the triglycerides and decrease in phospholipids in atretic follicles of control ovary were observed. However, after treatment, the triglycerides showed more increase and phospholipids showed more decrease after treatment with monocrotophos as compared to control. The group R showed moderate recovery in the level of triglycerides studied histochemically. Thus, it appears that this pesticide interferes with general lipids metabolism through oxidative stress resulting in reproductive toxicity. The group R showed moderate recovery.

KEYWORDS: Moncrotophos, Organophosphate pesticide, Triglycerides, Phospholipids, Ovary.

Introduction

Survival of any species depends on the integrity of its reproductive system. The toxic effects of drugs and environmental chemicals on the human reproductive system have become a major health concern; incidences of chemically induced germ cell damage and sterility appear to be on the increase[1,2]. Scott[3] has reported that some of the insecticides reduced the fertility and cause sterility in animals. Therefore, studies concerning the effect of pesticides on the reproduction are of recent interest. In this area, a progress has already been made with regard to male reproductive organs[4-7]. A start has been made to see the effect of pesticide on the female reproductive organs of mammals.

Many factors, both environmental and endogenous, can have detrimental effects on the female reproductive cycle and on the outcome of pregnancy. Environmental chemicals accidentally introduced into the human food chain have the potential of altering the endocrine system, if chemically present in the

diet[8-9]. Therefore, it is important to examine the acute effect of environmental chemicals on the reproduction potential of the animals. Present study was designed to see the histochemical effect of monocrotophos on ovaries of female albino rats in proestrous phase of estrus cycle.

MATERIAL AND METHODS

Healthy adult female albino rats of Wistar strain in proestrous phase of estrus cycle weighing 100-150 gm were obtained and divided into three groups TI, TII and R groups (8 rats in each group). LD^{50} of Monocrotophos was standardized on the basis of the dose calculated by Janardhan *et al*[10] and was found to be 14 mg/kg body weight. 1/5th of LD^{50} value of monocrotophos i.e. 2.8 mg/kg body weight was administered for 15 days to TI group and for 30 days to TII group. To the rats of R group, the same dose was given for 30 days and then the rats were kept on normal conditions i.e. without monocrotophos for 15 days. Another three group CI, CII and CIII (8 rats in same phase of estrus cycle in each group) were kept as corresponding controls for all the treatment groups. All the animals were kept on the commercial standard diet and tap water *ad libitum*. The weight of animals was recorded weekly.

At the end of the treatment period, blood of the female rat from each group in proestrous phase of estrus cycle was collected from the retro-orbital plexus under the light anaesthesia. Serum was prepared for estimation of triglycerides[11] and progesterone. Six rats from each of the treated and control rats lying in the same phase were sacrificed by cervical dislocation. Both ovaries were dissected out, weighed and the extraneous material was removed and ovaries were washed in saline. For histochemical studies, ovaries were fixed in formaldehyde calcium for 24 hrs. and processed for gelatin embedding according to the standard technique. The gelatin sections were cut by cryostat at 10 µ thickness and later on subjected to Sudan Black B (SBB) staining technique[12] and Acid Haematein (AH) [13] staining technique . Student's 't' test was employed for statistical analysis.

RESULTS AND DISCUSSION

The lipids were stained with SBB. In the control ovary, abundance of lipid granules was observed in primordial follicles, pre-antral follicles, antral follicles and corpora lutea. Stained sites were also observed in theca interna of pre-antral and antral follicles **(Pmgs. 1,5&6).**

Whereas in treated groups TI & TII, conspicuous changes had been reported in the amount and nature of sudanophilic lipids in the granulosa cells of atretic follicles. Accumulation of lipid droplets has been observed in the degenerating follicles **(Pmgs. 2).** These lipid droplets in the granulosa cells of atretic pre-antral and antral follicles in mammals are composed of triglycerides, cholesterol and some phospholipids. Actually atresia leads to excessive storage of neutral fats (triglycerides) **(Pmgs. 2)** and decrease in the amount of phospholipids[14] **(Pmgs. 8)**

Decrease in phospholipids and an increase in triglycerides observed during present studies, may be due to necrosis of theca interna cell **(Pmgs. 2& 8)**. Decreased phospholipids and an increase in triglycerides were also observed in the interstitial gland tissue present in stroma. These changes observed after MCP

treatment may be responsible for the impairment of steroidogenic synthetic role of theca interna cells[15,16] .

In atretic follicles and regressing corpora lutea also, the triglycerides showed more increase and phospholipids showed more decrease after treatment with monocrotophos as compared to control. **(Pmgs. 2 & 7)**. As stated by Guraya[17] in rat ovary, the most important change during the transformation of granulosa cells into the luteal cells is the development of diffused lipoproteins throughout the cytoplasm of the later. This change may be due to control mechanism in the biosynthesis of steroid hormones. This statement was supported with the work of electron microscopists[18,19], who have reported a granular endoplasmic reticulum throughout the cytoplasm in the luteal cells, theca interna cells and interstitial gland cell which confirm their function of steroidal biosynthesis. As the phospholipids decreased and triglycerides increased in the healthy corpora lutea after MCP treatment, it seems that luteal cells secrete less of steroidal hormone i.e. progesterone after MCP treatment. The decrease in progesterone estimated in blood serum after MCP treatment also confirms these observations. (Table 1)

Whereas in blood serum, the level of triglycerides showed a reduction of 7.28% and 12.53% ($p<0.05$) in TI and TII groups as compared to their controls respectively (Table2). Serum concentration of phospholipids showed a statistically significant increase of 9.92% in TI and 14.71% in TII (Table2). The decrease in concentration of plasma triglycerides paralled by an increase in trilglycerides observed during present studies are in strict agreement with the earlier studies made by various workers[20].

In R group, general lipids and triglycerides were moderately stained showing a lot of recovery in sections of ovary **(Pmg. 4)** . The serum level of triglycerides estimated was also recovered(Table 2). The recovery might be due to revival of reduced enzymatic activity responsible for detoxification of toxic agents in the liver of treated rats. Hence the workers who get exposed to organophosphorous sprays are required to take a brief period of rest to cope up with the any kind of reproductive abnormality and to minimize the danger of intoxication from organophosphorous pesticides including moncrotophos intoxication.

TABLE 1

Effect of Monocrotophos on progesterone in serum of female albino rats in proestrus phase of estrous cycle

Parameters	2.8 mg/kg body weight monocrotophos/day			
	30 days treatment (TII)		15 days recovery (R)	
	Contl	Exptl	Contl	Exptl
Progesterone	0.0035 ± 0.00004	0.0028 ± 0.00009***	0.0034 ± 0.00008	0.0031 ± 0.00004 **

The values are expressed as Mean ± S.D. (n=5)

*P<0.05; ** p<0.01, when the values are compared with respective controls.

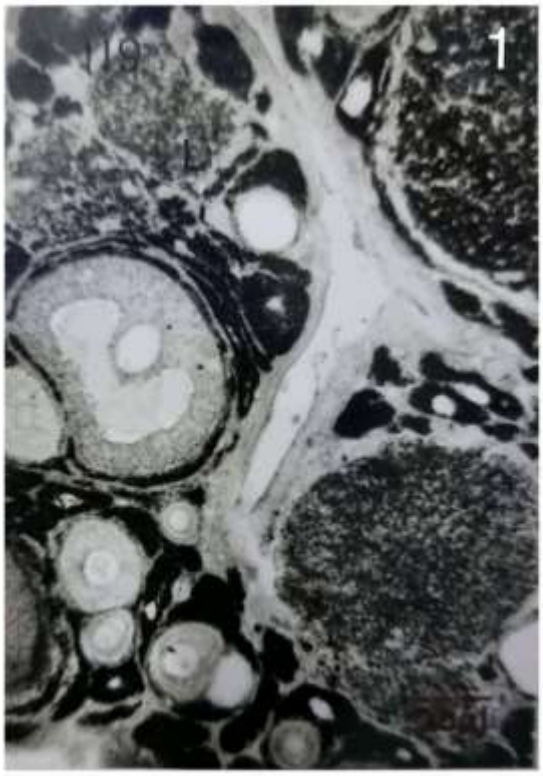
TABLE 2

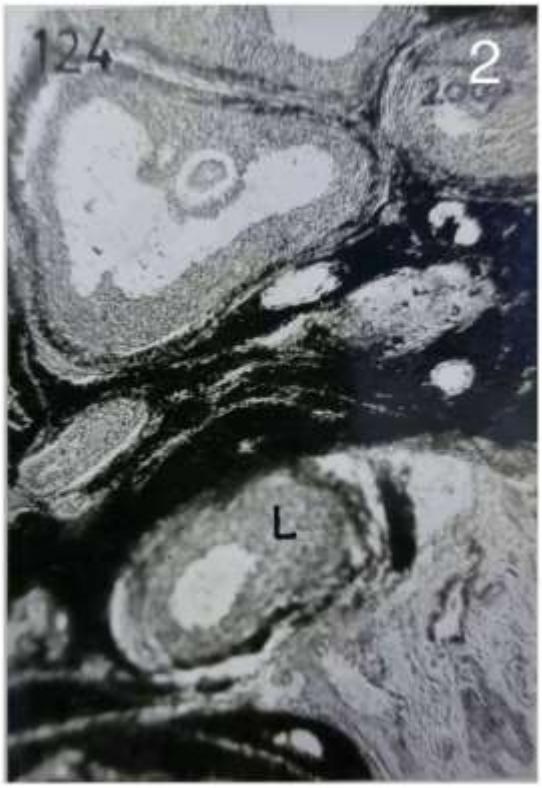
Effect of Monocrotophos on triglycerides in serum of female albino rats in proestrus phase of estrous cycle

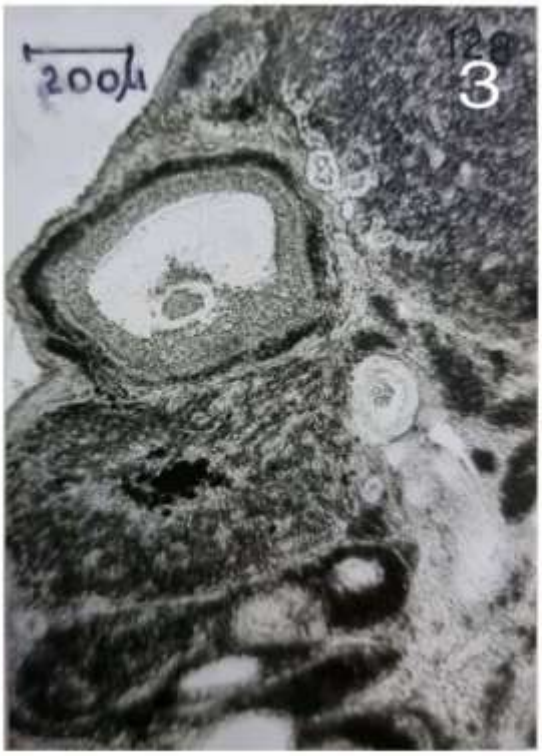
Parameters	2.8 mg/kg body weight monocrotophos/day					
	15 days treatment (TI)		30 days treatment (TII)		15 days recovery (R)	
	Contl	Exptl	Contl	Exptl	Contl	Exptl
Triglycerides	72.43 ± 8.67	67.15 ±7.93	74.67±6.82	65.31±9.14*	73.26±10.34	71.98 ±8.79*
% Change	(-) 7.28%		(-) 12.53%		(-) 1.74%	
Phospholipids	6.35. ± 0.69	6.98 ±0.84*	6.59 ± 0.72	7.56 ±0.37*	06.30 ± 0.69	6.72 ±0.28
	(+) 9.92%		(+) 14.71%		(+) 6.67%	

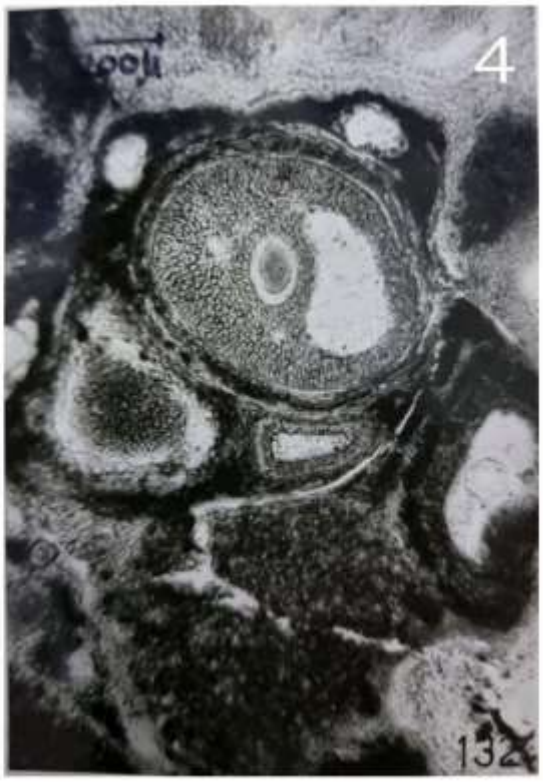
The values are expressed as Mean ± S.D. (n=5)

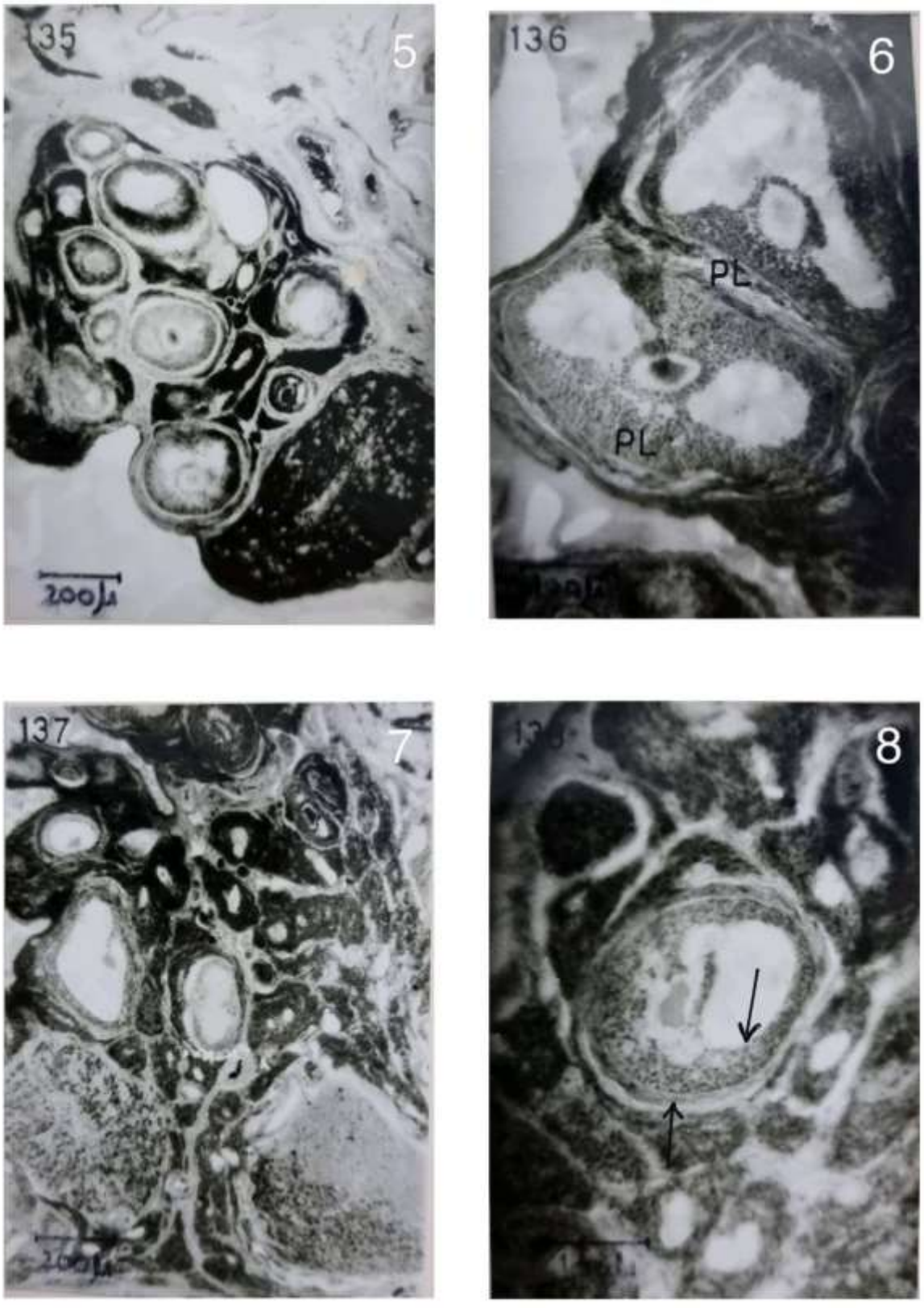
*P<0.05; ** p<0.01, when the values are compared with respective controls.


1
L


124
2
L


200μ
3


4
132



Pmg. 1 *T.S. control ovary showing the sites of lipid granules (L) in primordial follicles, pre-antral follicles, antral follicles and corpora lutea. Note the presence of stained sites of lipid granules in theca interna of pre-antral and antral follicles. FCa-PC/SBB.*

Pmg. 2 *T.S. ovary of TI group showing increase I staining at the sites of lipid granules in theca interna and granulosa cells of antral follicle, graffian follicle and atretic follicle (L). FCa-PC/SBB.*

Pmgs.3 *T.S. ovary of TII group showing an increase in staining at the sites of lipid granules in theca interna of graffian follicle and lutein cells of corpus luteum. FCa-PC/SBB.*

Pmg. 4 *T.S. ovary of R group showing decrease in sites of lipid granules in granulosa cells and theca interna of antral follicle. FCa-PC/SBB.*

Pmg. 5 *T.S. control ovary showing the sites of phospholipids in granulosa cells and theca interna of pre-antral, antral and graffian follicles. FCa-PC/AH.*

Pmg. 6 *T.S. control ovary showing the sites of phospholipids (PL) in granulosa cells and theca interna of antral follicle. FCa-PC/AH.*

Pmg. 7 *T.S. ovary of TI group showing decrease at the sites of phospholipids in granulosa cells and theca interna of antral follicles and lutein cells of corpus luteum. FCa-PC/AH.*

Pmg. 8 *T.S. ovary of TI group showing decrease at the sites of phospholipids in theca interna (arrow) and granulosa cells (arrow) of antral follicle. FCa-PC/AH.*

ACKNOWLEDGEMENTS

The author is grateful to the Chairman, Department of Zoology, Panjab University, Chandigarh (India) for providing necessary laboratory facilities and to University Grants Commission, New Delhi, for financial help.

REFERENCES

1. Lucier GW, Lee IP, Dixon RL. Effects of environmental agents on male reproduction. In: The Testis, Advances in Physiology, Biochemistry and Function (Johanson, A.D. and Gomes, W.R., ed.) Vol. IV., Academic Press, Inc., New York. P 578-98.
2. Habison RD and Dixon RL. Proceedings of symposium on target organ toxicity: Gonads (reproductive and genetic toxicity). Environ Hlth Perspect. 1978; 24: 1-128.
3. Scott R. Reproductive hazards. Jab Safety Hlth. 1978; 6: 7-13.
4. Whorton D, Krauss RM, Marshall S, Milby TH. Infertility in male pesticide workers. Lancet. 1977; 2: 1259-1261.
5. Soliman AA and el-Zalabani LM. Impairment of spermatogenesis by organophosphorus pesticides. Bulletin of the Alexandria Faculty of Medicine. 1981; 17: 125.
6. Salem MH, Abo-Elezz Z, Abd- Allah GA, Hassan GA and Shaker N. Effect of organophosphorus (Dimethoate) and pyrethroid (Deltamethrin) pesticides on semen characteristics in rabbits. J Environ Sci Hlth., Part B. Pestic. Food Contam Agric Wastes. 1988; 23(3): 270-290.
7. Bhunya SP and Behera BC. Mutagenicity assay of an organophosphate pesticide, monocrotophos in mammalian in vivo test ststen. Cytologia. 1988; 53: 801-807.
8. Butcher RL and Page RD. Introductory remarks: Environmental and endogenous hazards to the female reproductive system. Environ Hlth Perspec. 1981; 38: 35-37.
9. Krieger RI . Handbook of Pesticide Toxicology . 2nd Ed. Vol I, Academic Press, Florida. 2001; P 375-423.

10. Janardhan A, Bhaskar Rao A, Sisodia A. Species variation in acute toxicity of monocrotophos and methyl benzimidazole carbamate. Ind J Pharmacol. 1986;18:102-103

11. Van Handel E and Zilversmit DB. Micromethod for direct determination of serum triglycerides. J Lab Clin Med.1957;50:152.
12. Baker JR. Improvement in Sudan Black B technique. Quart J Micr Sci. 1956; 97:621.
13. Baker JR. Cytological Techniques. 2nd Ed., Methuen, London, 1945.

14. Guraya SS. Biology of Ovarian Follicles in Mammals. Springer – Verlag Berling, Heidelberg, New York 1985.
15. Fortune JE and Armstrong DT. Androgen production by theca and granulosa isolated from proestrous rat follicles. Endocrinology. 1977; 100:1341-1347 (Cited from Uilenbroek and Richards 1979).
16. Fortune JE and Armstrong DT. Hormonal control of 17 β-estradiol biosynthesis in proestrous rat follicles: Estradiol production by isolated theca versus granulosa. Endocrinology. 1978; 102:227-235.
17. Guraya SS. Comparative studies on the histochemical features of ovarian compartments in the rat and golden hamster, with special reference to steroid hormone synthesis. Acta anat. 1972; 82:284-304
18. Goodman P, Latta JS, Wilson RB and Kadia B. The fine structure of saw lutein cells. Anat. Tec. 1968; 161:77-90 (Cited from Guraya, 1972)
19. Davies J and Broadus CD. Studies on the fine structure of ovarian steroid-secreting cells in the rabbit. I. The normal interstitial cells. Am J Anat. 1968; 123: 441-474.
20. Bandyopadhyay U, Das D, Banerjee RK. Reactive oxygen species: oxidative damage and pathogenesis. Curr Sci. 1999; 77:658-66.