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Selection of Gamma Irradiation Dose for Sterilising Eye Make up Preparations

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IN THE PRESENT study, radioresistant bacterial strains isolated from the dried mixed populations of bacteria of, eye make up preparations were used. The mixed population of bacterial strains from eye shadow, eye mascara and eye liner, survived the radiation doses of 5-14 kGy, 5-6 kGy and 4-11 kGy, respectively. Dose survival curves of the radioresistant bacterial strains exhibited exponential response, except *Bacillus cereus* which showed non exponential response towards gamma radiation. The D_{10} values were in the range of 0.7-1.8 kGy, 0.8-1.0 kGy and 0.7-1.4 kGy for the strains from eye shadow, eye mascara and eye liner, respectively. The sterilization doses for the eye make up products were determined from the dose response curves. The maximum sterilization doses were found to be 16.0, 8.3 and 12.3 kGy for sterilization of the eye make up products at the same mentioned order.

Keywords : Eye make up preparations, Mixed population, Gamma radiation, Sterilization.

The need to control microbiological contamination of all products for human use and consumption, has been of considerable concern to manufacturers. Modern food, pharmaceutical, cosmetic and toiletry industries strive for high microbiological standards to protect their products from spoilage on the one hand, and their consumers from infection, on the other hand. Unlike foodstuffs, which are usually kept refrigerated or thrown away after a few days, a much longer shelf life is expected for personal care products (Razem *et al.*, 2003).

A standard hygiene control system is vital in order to lower the level of contamination to safe level. Ionizing radiation is used for decontamination purposes. The pharmaceutical industry has been leading the way in the change of attitudes from the quality control (QC) to the quality assurance (QA) approach. Treatment by irradiation is a particularly suitable measure to be integrated into a comprehensive (QA) approach in the processing of biological materials for microbial safety. Ionizing radiation is a universal, nonselective biocidal agent; it acts on all forms of life, in all parts of an irradiated volume; it can be delivered in precise portions of dose; and there is a well-defined relationship between the degree of killing of microorganism and irradiation dose (Clegg, 1988 and Razem, 2001).

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The most important consideration in selecting a particular sterilization dose is the destruction of microorganisms with no alteration in the product properties. Thus knowledge on the type and number of organisms per unit product prior to sterilization is of considerable value (Roushdy *et al.,* 1999).

The present study was elucidated to determine the radiation doses for sterilizing eye make up preparations sold in Egypt.

Materials and Methods

Microorganisms

A total of seventy seven (77) bacterial contaminants were previously isolated from eye make up products obtained from the local market (38 bacterial isolates from eye shadow samples, 23 from eye mascara samples and 16 from eye liner samples) in our previous study (El Bazza *et al*., 2009) . The bacterial isolates were purified and identified. They were maintained on nutrient agar, stored at 4ºC and subcultured monthly on the same medium.

Eye make up samples

A total of fifty seven (57) eye make up samples were collected from the local market and bacterial contamination was reported in our previous study (El-Bazza *et al*., 2009): 26 eye shadow samples manufactured by 6 different companies (A, C, D, E , F and G), 20 eye mascara samples manufactured by 5 different companies (A, H, I, J and K) and 11 eye liner samples manufactured by 3different companies(A, H and I).

Chemicals

Peptone, Lab-Lemco (Oxoid), Yeast extract (BBL) and Agar-Agar (Difco) were used. Other chemicals used in the present study were of the reagent grade.

Gamma irradiation facility

The Cobalt-60 source (Indian Gamma cell 4000A) located at the National Centre for Radiation Research and Technology (NCRRT), Nasr City, Cairo, Egypt was utilized as the radiation resource in the present investigation. The dose rate was 1.00424 kGy / sec at the time of experiments.

Dose survival curve of the bacterial organisms to gamma radiation

The bacterial strains isolated in a pervious study from samples representing each manufacturing company were heavily cultured on nutrient agar plates and the grown colonies were used to prepare test pieces according to Christensen & Holm (1964) and Christensen & Kristensen (1981). A twenty four hour of each bacterial culture was used for surface inoculation on nutrient agar plates. After incubation at 35 ± 2ºC for 48 hr, the bacteria was scrapped off by using sterile glass slide and transferred into a small porcelain mortar, horse serum broth was added dropwise to the mortar and then homogenized to have a thick suspension. The resulting dense suspension of microorganisms was distributed as separate spots, each 20 µl, on sterile polyethylene foil (pre sterilized at 25 kGy) and dried at room temperature on a laminar air bench. The dried spots were then covered with another sterile polyethylene sheet and the two layers were heat-sealed in squares each containing one spot (using Impulse sealer Tisw 300 waat 430-450 W, Tiwan). The test pieces, in triplicates, were exposed at room temperature to gamma radiation doses ranging from 2.0 to 15.0 kGy.

Isolation of the radioresistant bacterial organisms

The irradiated test pieces were aseptically removed from the polyethylene bags and each was transferred into 10 ml of sterile saline {0.89(w/v) % $NaCl/H_2O$}, shaken vigorously and ten fold serial dilutions in the same solution were made. Aliquots of 0.1 ml from the homogenous suspension were used for surface inoculation on nutrient agar plates (peptone, 5g; sodium chloride, 1.0g ; yeast extract, 3.0g, Lab Lemco, 1.0g; Agar, 20g and distilled water, 1000 ml). The plates were incubated at 35±2ºC and examined daily for a maximum period of one week and the growth was examined macroscopically. The bacterial isolates from each type of the eye make up product, which could survive the highest radiation doses under treatment, were further cultured on nutrient agar. The radioresistant isolates were identified according to Cowan & Steel's (1985).

Dose response curve of the selected radioresistant bacterial strains

Test pieces for each radioresistant bacterial strain were prepared as mentioned before. Triplicate test pieces for each strain were exposed at room temperature to gamma radiation doses suitable for each radioresistant strain. Non irradiated test pieces served as control. After irradiation, the number of surviving colonies was counted and the mean of triplicate plates for the test pieces was reported as cfu ml^{-1}.

The survival curves were constructed by logarithmic plotting of the number of survivors versus the radiation doses in kGy in linear scale.

The D_{10} value which is the measure for the radiation resistance of the microorganisms was obtained according to Schmidit & Nank (1960).

Determination of the bioburden of eye make up samples

The total bacterial counts were determined for eye make up samples representing the different manufacturing companies. One ml or g of each sample was mixed with 9 ml sterile dilluent, 0.1% (w/v) peptone water, containing 0.1% (v/v) Tween 80 (Behravan *et al.,* 2005). Ten fold serial dilutions were made and aliquots of 0.1 ml were taken from each appropriate dilution and spread on solidified nutrient agar. The plates were incubated at 35±2ºC for 4 days. The total bacterial count was determined. The mean of the total bacterial counts for each group of samples representing a manufacturing company (Bioburden) was then recorded.

Calculation of the sterilization doses

The radiation sterilization dose for each product was calculated based on the average initial count of the contaminating microorganisms (bioburden) on this product and the radiation resistance (D_{10} value) of the most radiation resistant contaminant and the sterility assurance level (SAL) required for this product (Christensen *et al.*, 1967 a, b ; Miller & Berube, 1978 and USPXXI, 1985).

For determination of the sterilization dose of certain product, the survival curve of the most radiation resistant bacterial strain was used. A parallel curve to that of the radiation resistant microorganisms is drawn starting at initial count equals the average bioburden on the product wanted to be sterilized. The drawn curve is extrapolated to the negative side of the y-axis. For the eye make up preparations (10^{-6}) is the sterility assurance level. To get the sterilization dose for this product, a parallel line to the x-axis is drawn starting at log cycle (-6) on the negative side of the y-axis until it meets the extrapolated part of the curve, then a parallel line to the y-axis is drawn until it meets the x-axis to give the required sterilization dose which realizes the sterility assurance level of one per million (10^{-6}). This is for straight line survival curves, but if the survival curve of the most radiation resistant microorganism is shouldered, the size of the shoulder was defined by the "intercept" on the 100% survival axis (quasi-threshold dose, Dq). So, (Dq) was added to the determined dose value (Roushdy *et al.*, 1999).

Applicability of radiation sterilization dose

The calculated radiation sterilization doses for eye make up samples were applied on the most contaminated samples of each individual company. The probability of sample contamination was studied by determination of the viable count on the samples after application of the calculated sterilization doses, as mentioned before.

Results and Discussion

Although, the use of irradiation decontamination is not very old, it has found its way into many applications. However, there is still much to be investigated to improve the efficiency and to find an ideal procedure which radiation can decontaminate or sterilize items with minimal alteration of their components (Tilquin, 1991; Sainz *et al.*, 1999 and Salih, 2001). Ionizing radiation is very practical in achieving sterility without noticeable changes in the appearance or structure of the irradiated material. Nevertheless, the technology is still limited in use because of unavailability of suitable radiation facilities mainly in some of developing countries (Salih, 2001).

In the present investigation an acceptable level of sterility of eye make up products was attained by the use of γ-irradiation. The dried preparations of the mixed population of previously isolated bacterial strains from eye make up samples (38 strains of eye shadow, 23 strains of eye mascara and 16 strains of eye liner) representing some individual companies, were used in this study. The isolated radioresistant bacteria after exposure of the mixed populations to gamma irradiation (2.0 – 20 kGy) were shown in Table 1. The resistant bacteria were identified as *Staphylococcus aureus, Bacillus megaterium* and *Staphylococcus epidermidis*, from eye shadow ; *Staphylococcus aureus* and *Staphylococcus epidermidis* from eye mascara and three strains of *Bacillus cereus, Staphylococcus epidermidis* and *Staphylococcus aureus*, from eye liner. Those bacterial isolates survived from 5-14, 5-6 and 4-11, kGy, respectively.

TABLE 1. Evaluation of the radio-resistant microbial strains after exposure of the mixed bacterial population to gamma radiation (kGy).

Eye make up	Co.	Mixed population	No. of strains	Radio-resistant strain	Sub D* kGy	D_{10}
Eye shadow	A	*Staphylococcus aureus*	5	*Staphylococcus aureus*	6	0.9
	C	*Bacillus megaterium* and *Staphylococcus aureus*	5 and 5	*Bacillus megaterium*	14	1.7
	D	*Staphylococcus aureus* and *Klebsiella pneumonia*	4,4	*Staphylococcus aureus*	7	0.95
	E	*Staphylococcus aureus*	4	*Staphylococcus aureus*	6	1.0
	F	*Staphylococcus epidermidis*	5	*Staphylococcus epidermidis*	5	0.7
	G	*Bacillus megaterium* and *Staphylococcus aureus*	3 and 3	*Bacillus megaterium*	13	1.8
Total		--	38	*6*	--	--
Eye mascara	A	*Staphylococcus aureus*	5	*Staphylococcus aureus*	6	1.0
	H	*Staphylococcus aureus*	5	*Staphylococcus aureus*	6	1.0
	I	*Staphylococcus aureus* and *Staphylococcus warneri.*	3 and 3	*Staphylococcus aureus*	6	0.9
	J	*Staphylococcus aureus*	3	*Staphylococcus aureus*	6	1.0
	K	*Staphylococcus epidermidis*	4	*Staphylococcus epidermidis*	5	0.8
Total			23	*5*	--	--
Eye liner	A	*Bacillus cereus* and *Staphylococcus aureus*	5 and 5	*Bacillus cereus*	11	1.4
	H	*Staphylococcus epidermidis*	4	*Staphylococcus epidermidis*	5	0.8
	I	*Staphylococcus aureus*	2	*Staphylococcus aureus*	4	0.7
Total			16	*3*	--	--

Sub D* kGy: Sub-lethal radiation dose.

In this respect, Christensen & Kristensen (1981) isolated radioresistant bacterial strains by exposing dried test pieces containing mixed population of bacteria to gamma irradiation dose levels from 15-50 kGy, While Ashour *et al.* (1990) found twenty radioresistant bacterial strains survived gamma radiation at 18-20 kGy of which nineteen were identified as *Bacillus* sp. and one as *Micrococcus roseus.*

Radiation resistance of microorganisms can be associated with the D_{10} value, the dose of radiation required to reduce the microbial population by 90% (Miller & Berube , 1978).

In the present investigation, the D_{10} values of the radioresistant bacterial strains (Table 1) were calculated and found to range from 0.7–1.8 kGy, 0.8–1.0 kGy and 0.7–1.4 kGy for the strains isolated from eye shadow, eye mascara and eye liner samples, respectively. In this field Bochkarev *et al.* (1978) examined the radiation sensitivity of about 8000 strains of Gram positive microorganisms of dried culture preparations, of which 1500 strains were of *Staphylococci*; 30% were found to have D_{10} value of 0.1-1.0 kGy and the 70% had D_{10} value of 2.0 kGy.

In another study, Ashour *et al.* (1993) examined the response of *Bacillus sterothermophillus, B. pantothenticus, Bacillus licheniformis, B. coagulans, Bacillus laterospores, Bacillus cereus, Bacillus megaterium, Bacillus pumilis* E601 towards gamma radiation and determined the D_{10} values for these strains ranged from 2.33 to 4.1 kGy . On the other hand, Kotiranta *et al.* (1999) reported that four strains of *Bacillus cereus* were sensitive to gamma radiation with D_{10} value 0.4 kGy.

The D_{10} values of *Staphylococcus aureus*, *Bacillus cereus* and *Bacillus sphaericus* were found to be 0.37, 1.0 and 1.4, respectively (Farrag *et al.*, 2000 and El-Fouly *et al*., 2000). The dose response curve of *Bacillus cereus* showed exponential response curve and the D_{10} value was calculated as 0.9 kGy (El-Tablawy & El-Hifnawi, 2009).

On the other hand, Miller & Berube (1978) examined the effect of radiation on microorganisms and demonstrated two typical dose response curves for microorganisms. Both showed exponential killing, but the one that reflect the response of most organisms showed sensitivity to the radiation. Some organisms are more resistant. The authors were concerned with the resistant organisms than with the sensitive ones.

In the present study, dose response curves of the selected different radioresistant bacterial strains were constructed. The results (Fig. 1 to 8) showed a decrease in the number of survivors with the increase of the radiation dose for the tested radioresistant strains. Gram positive cocci and *Bacillus megaterium* exhibited exponential rate of death towards gamma radiation and this is manifested by straight line curves (Fig. 1-5 and 7-8). This was in agreement with those reported by other investigators who found that some *Bacilli* such as *B.*

megaterium and *B. pumilis*, also Gram positive cocci had an exponential rate of death (El-Schafei,1982; Ashour *et al.*, 1993; El-Fouly, 2000; Salih, 2001; Bashandy, 2005 and El-Tablawy & El-Hifnawi, 2009). Only *Bacillus cereus* (Fig. 6) from eye liner sample exhibited non-exponential rate of death towards gamma irradiation and this is manifested as straight line curve proceeded by an initial shoulder.

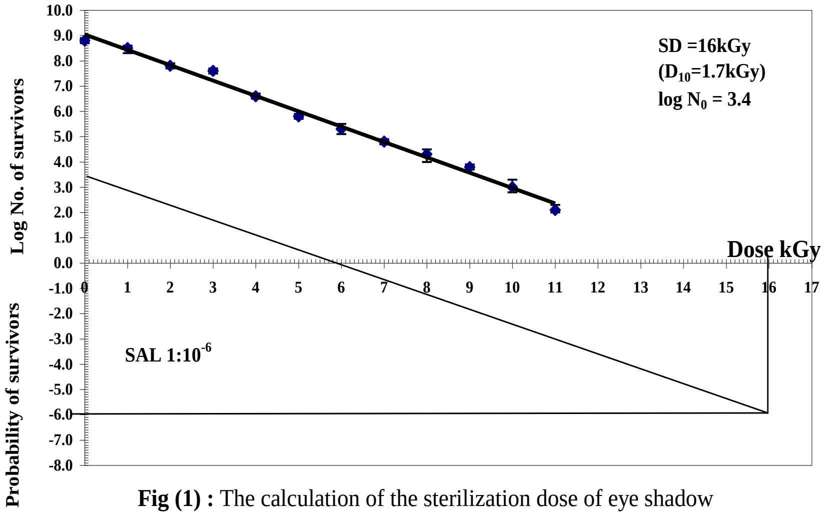

Fig.1. The calculation of the sterilization dose of eye shadow samples Co. (C) using *Bacillus megaterium* (D_{10}=1.7kGy) as the most radioresistant isolate.

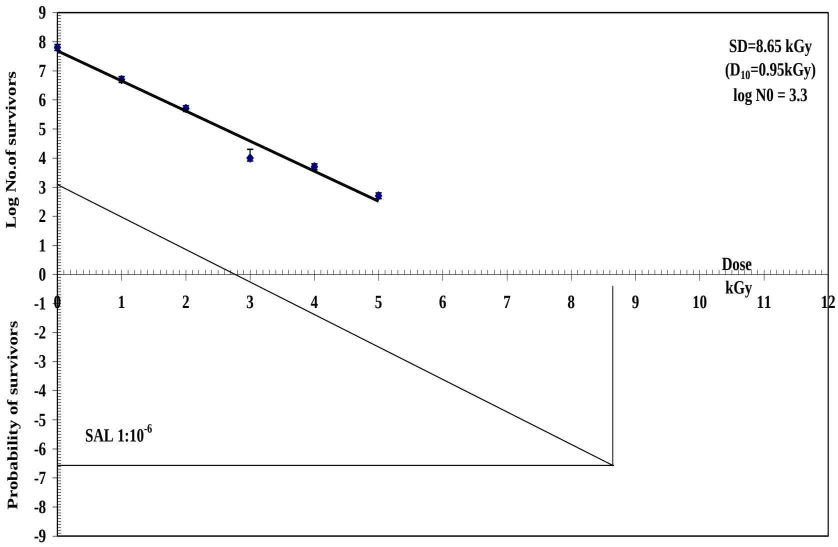

Fig. 2. The calculation of the sterilization dose of eye shadow samples Co. (D) using *Staphylococcus aureus* (D_{10}=0.95 kGy) as the most radioresistant isolate.

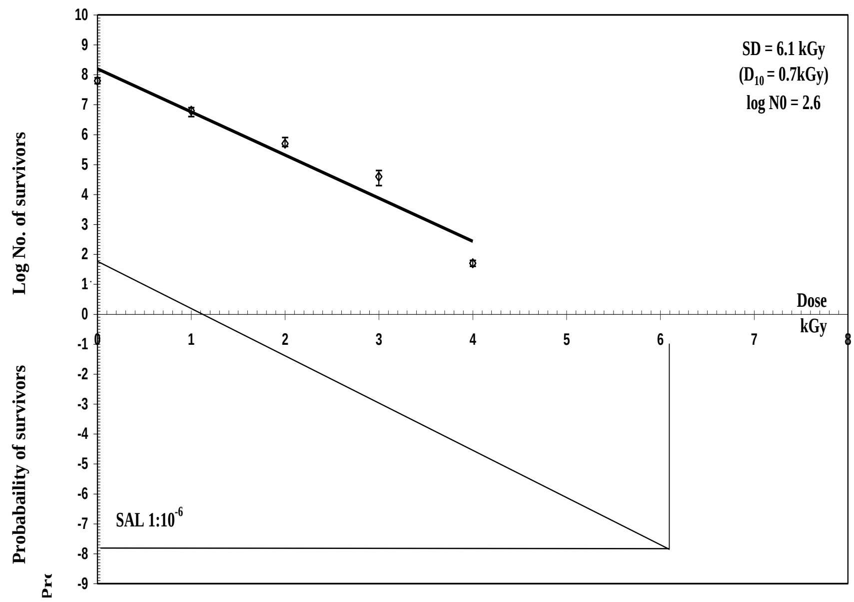

Fig. 3. The calculation of the sterilization dose of eye shadow samples Co. (F) using *Staphylococcus epidermidis* (D_{10}=0.7 kGy) as the most radioresistant isolate.

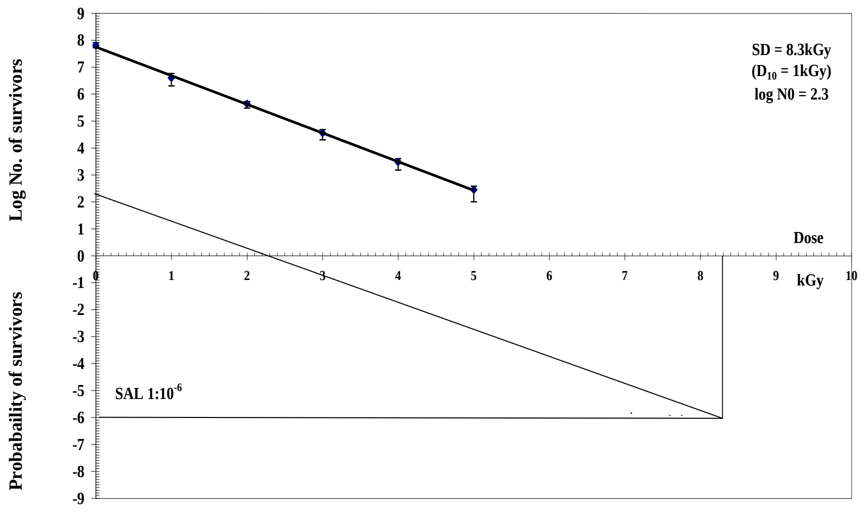

Fig. 4. The sterilization dose calculation of eye mascara samples Co. (A) using *Staphylococcus aureus* (D_{10}=1 kGy) as the most radioresistant isolate.

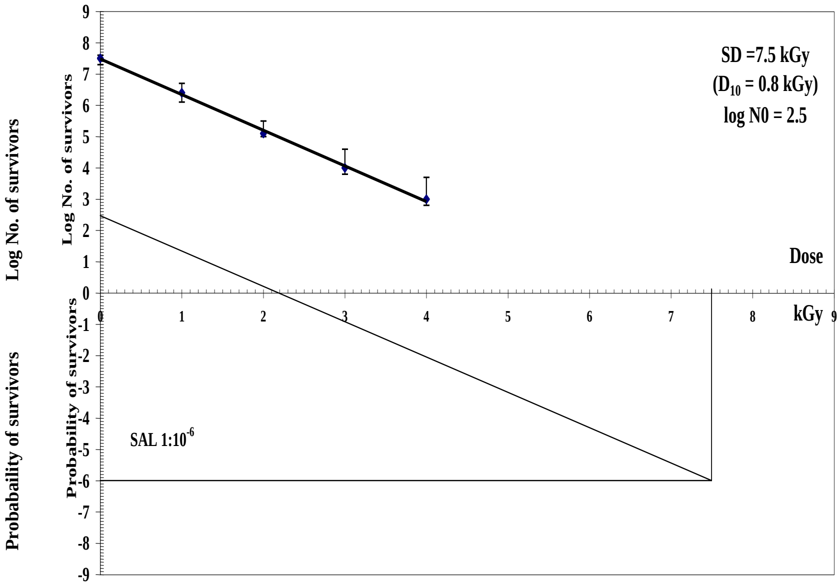

Fig. 5. The sterilization dose calculation of eye mascara samples Co. (K) using *Staphylococcus epidermidis* (D_{10}=0.8 kGy) as the most radioresistant isolate.

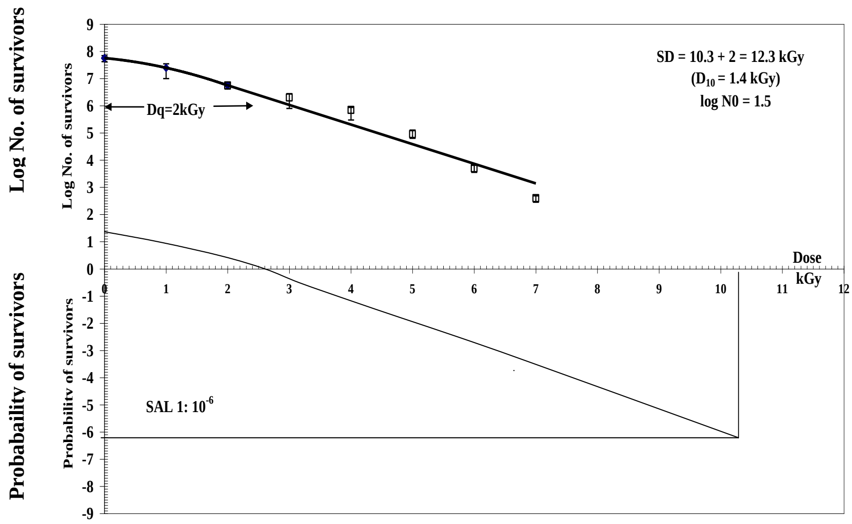

Fig. 6. The calculation of the sterilization dose of eye liner samples Co. (A) using *Bacillus cereus* (D_{10}=1.4 kGy) as the most radioresistant isolate.

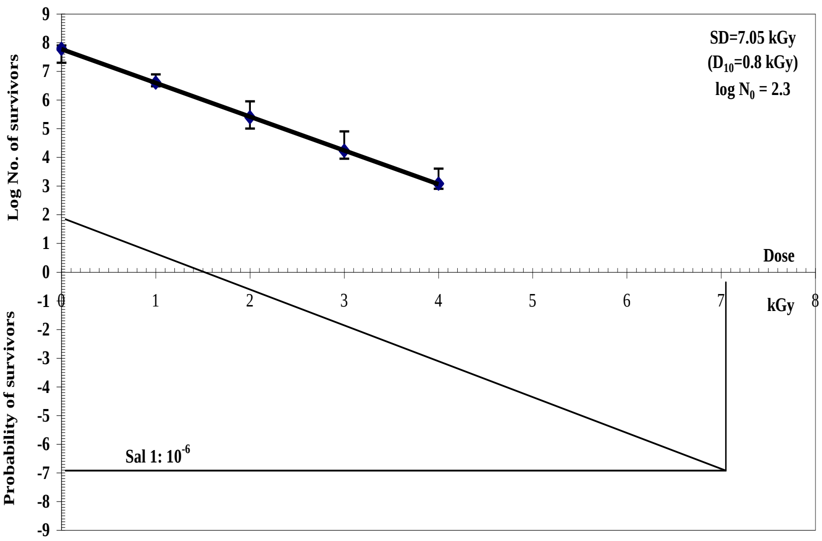

Fig. 7. The calculation of the sterilization dose of eye liner samples of Co. (H) using *Staphylococcus epidermidis* (D_{10}=0.8 kGy) as the most radioresistant isolate.

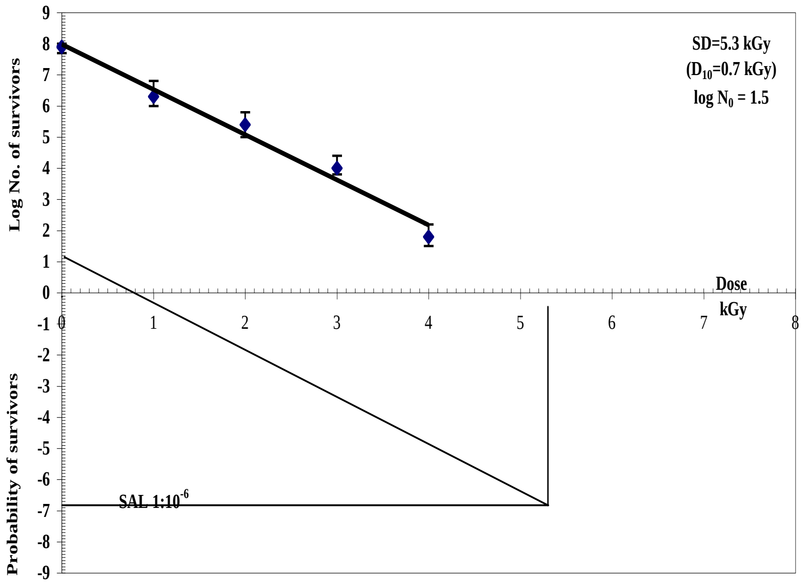

Fig. 8. The calculation of the sterilization dose for eye liner samples of Co. (I) using *Staphylococcus aureus* (D_{10}=0.7 kGy) as the most radioresistant isolate.

Similar results were obtained by Ashour *et al.* (1993) who obtained this response with some *Bacillus* spores such as *B. pantothenticus, B. stearothermophilus, B. circulans, B. coagulans* and *B. laterosporus* and disagree with El-Fouly *et al.* (2000) and Abostate *et al.* (2006) who found that *Bacillus cereus* showed an exponential response towards gamma radiation.

The microbiological quality (sterility assurance level or SAL of 10^{-6}) of the finished medical product should be a maximum of one contaminant per one million sterilized products (Christensen *et al.*, 1967 a, b). The United State Pharmacopoeia (1985) stated that the choice of the sterilization dose should be determined by the knowledge of the microbiological bioburden (types and numbers) and the nature of the article to be sterilized.

The dose of ionizing radiation, the microbial contamination load and the degree of radiation resistance of the contaminants are among the factors which determine the efficiency of radiation sterilization (Miller & Berube, 1978; USPXXI, 1985 and Salih , 2001) .

The sterilization doses in the present study were calculated by applying the survival curves of the radiation resistant isolates (with the highest D_{10}- value). The calculated sterilization doses for eye shadow of Co. (C, D and F) were 16, 8.7 and 15.5 kGy, respectively. While the doses for eye mascara of Co. (A and K) were 8.3 and 7.5 kGy. Also, the sterilization doses for eye liner samples of Co. (A, H and I) were 12.3, 7.05 and 5.3 kGy, respectively (Table 2).

The results revealed that the maximum doses obtained in the present investigation are 16 kGy, 8.3 kGy and 12.3 kGy for sterilization of the studied eye shadow, eye mascara and eye liner, respectively.

The highest sterilization dose obtained for eye liner samples was high compared to those reported by Razem *et al.* (2003) which was found to be 1.9, 2.2 and 5.3 kGy for eye liner pencils, however, it should be taken into consideration that in the present study liquid eye liner samples were investigated. It is worthy to clarify, in our study that the contaminants were Gram positive bacteria while; the contaminants in the foregoing literature were mainly Gram-negative bacteria and molds. Also, the sterilization doses for powdered eye shadow were higher than eye mascara and eye liner; this is may be attributed to the higher bioburden of the microbial contaminants in the eye shadow samples and their relatively higher microbial resistance to gamma radiations (D_{10}-values).

TABLE 2. Gamma sterilization doses of the eye make up products (different manufacturing companies).

Eye make up	Company	N_o (a)	Log N_o	Radio-resistant strain	SD(b) kGy
Eye shadow	A	280	2.4	*Staphylococcus aureus*	8.1
	C	2400	3.4	*Bacillus megaterium*	16
	D	2000	3.3	*Staphylococcus aureus*	8.65
	E	500	2.7	*Staphylococcus aureus*	8.7
	F	380	2.6	*Staphylococcus epidermidis*	6.1
	G	400	2.6	*Bacillus megaterium*	15.5
Eye mascara	A	210	2.3	*Staphylococcus aureus*	8.3
	H	128	2.1	*Staphylococcus aureus*	8.1
	I	267	2.4	*Staphylococcus aureus*	7.5
	J	127	2.1	*Staphylococcus aureus*	8.1
	K	335	2.5	*Staphylococcus epidermidis*	7.5
Eye liner	A	36	1.5	*Bacillus cereus*	12.3
	H	200	2.3	*Staphylococcus epidermidis*	7.05
	I	35	1.5	*Staphylococcus aureus*	5.3

(a) N_o= Average bioburden on the samples.
(b) SD=The calculated sterilization doses.

It is worthy to mention that, the calculated sterilization doses in the present study were applied on the heavily contaminated samples representing each individual company (7 eye shadow samples from 6 manufacturing companies, 6 eye mascara samples from 5 companies and 2 eye liner samples from 2 companies). No microbial contaminants were detected after the irradiation process (Table 3).

TABLE 3. Irradiation of the most heavily contaminated samples with the determined sterilization doses.

Eye make up	Company	Samples No.[(a)]	Dose (kGy)	Bacterial contamination count (c.f.u/g or ml)	
				B.ir[(b)]	A.ir[(c)]
Eye shadow	A	3	8.1	$8x10^2$	-
	C	1	16	$4 x10^3$	-
	D	2	8.65	$5 x10^3$	-
	E	1 4	8.7	$6 x10^2$ $5 x10^2$	-
	F	3	6.1	$7 x10^2$	-
	G	3	15.5	$6 x10^2$	-
Eye mascara	A	5	8.3	$7 x10^2$	-
	H	2	8.1	$3 x10^2$	-
	I	1 2	7.5	$3 x10^2$ $3 x10^2$	-
	J	3	8.1	$3 x10^2$	-
	K	1	7.5	$5 x10^2$	-
Eye liner	A	4	12.3	$0.5 x10^2$	-
	H	4	7.05	$3 x10^2$	-
	K	3	3.25	-	-

(a) : The heavily contaminated samples. (c) A.,ir: After irradiation.
(b) B.,ir: Before irradiation . - : No detectable growth.

In this respect, Boegl (1985) reported that the early work on pharmaceuticals and cosmetics relied on the application of 25kGy dose for sterilization. However, such a high dose was unrealistic as much as it causes wasteful damaging to products. The 25 kGy dose assumed that contamination levels of the pre-sterilized products were high of 10^3.

Conclusion

The gamma radiation technology can offer the process of sterilization of cosmetics as a mean of achieving a higher standard of microbiological safety limits, decreasing the bioburden on cosmetics and eliminating pathogenic microorganisms.

The microbial isolates from the eye make up are fortunately, radiation sensitive microbes, which mean that sterilization doses were also lower than those reported in the case of pharmaceuticals. Sterilization doses lower than 25 kGy are considered as a benefit for the manufacturers who want to use the radiation technology decontamination.

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إختيار الجرعه الأشعاعيه المناسبة لتعقيم بعض مستحضرات تجميل العين

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فى هذه الدراسة تم عزل الميكروبات المقاومة للإشعاع من كل خليط العزلات البكتيرية (السابق عزلها من مستحضرات تجميل العين) والتى تستطيع أن تعيش عند مستويات (٥ – ١٤ كيلو جراى ، ٥ – ٦ كيلو جراى ، ٤-١١ كيلو جراى) من مستحضرات ظل الجفون ، مجمل الرموش ومخلوط العين على التوالي.

وقد أظهرت منحنيات الإستجابة للإشعاع لهذه العزلات علاقة خطية ما عدا خليط *الباسيلس سيرس* الذى بدأ بعلاقة غير خطية أعقبها علاقة خطية. وقد تم تقديـر الجـرعة (**د**.$_{١٠}$) ووجـد أنـها ٠,٧ ـ ١,٨ ، ٠,٨ ـ ١ ، ٠,٧ ـ ١,٤ كيلـو جراى , على التوالى.

كما تم إستخدام منحنيات الإستجابة للإشعاع لهذه العزلات فى تقدير الجرعة اللازمة لتعقيم مستحضرات تجميل العين و وجدت أنها ١٦ ، ٨,٣ ، ١٢,٣ كيلو جراى على التوالى بالنسبة للمستحضرات الثلاثة.