Effect of Pinholes on Sterile Barrier Properties

Earl T. Hackett, Jr.
Research Associate
DuPont Medical Packaging
Presented at HealthPak, March 2001, in St. Petersburg, Florida

Abstract

The probability of a particle penetrating a defect in a package is a function of the particle and defect size as well as the flow rate through the defect. Equations describing particle motion in an air stream are used to estimate the probability of particles penetrating a package defect, and these results are compared with experimental data. The range of pressure differentials experienced during the shipment of sterile devices is examined and the overall significance of pinholes in sterile packaging is discussed.

Background Information

Over the last 20 years many studies have been performed to determine the effect of pinholes on the microbial barrier characteristics of various package designs. These studies have been prompted by the proliferation of prepared packaged foods and the increased awareness of the threat of microbial contamination of medical instruments. These tests have been performed using a wide range of protocols and the results have been contradictory. Some laboratory tests have shown that sub-micron holes will allow contamination, while studies of commercial packages with holes larger than 10 µm were found to be free of microbial contamination.[1,2,3,4,5,6,7]

In cases where small leaks have allowed device contamination, the test protocol has employed very high challenge concentrations and test conditions well beyond the environment a package would see in normal commerce. In these cases, the microorganism may be used as a probe to detect a leak rather than to determine the effect of the leak on the package performance.

A variety of techniques are used to assure the integrity of medical packaging. All test methods have a minimum size defect that can be detected. Test methods commonly in use today have size limits in the range of 25 µm to 75 µm diameter, sizes large enough to allow microbial penetration. Unresolved is the question, "What is the effect of small leaks, below the limits of detection, on package integrity in the normal commercial environment?" To determine the effect of any given leak on package integrity, consideration must be given to the package design, including head space, materials of construction and the characteristics of any breathable membrane.

Pressure Differentials

The flow of gas (and the particles it contains) into a package may be the most serious threat to the sterility of a packaged device. The level of threat is dependent upon the volume of gas transported and the conditions under which that transport occurred. A major cause of pressure differential during transport is the ascent and descent of transport aircraft. Figure 1 shows three barograph traces taken during air transport operations. The

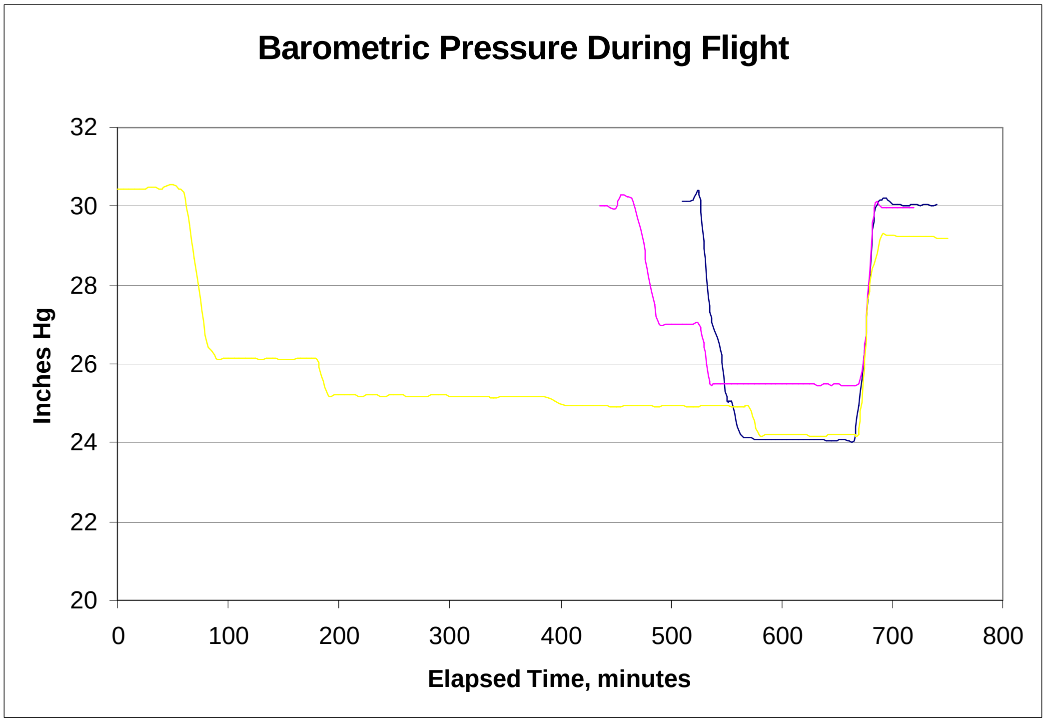

Figure 1: Barograph Traces During Commercial Aircraft Flights

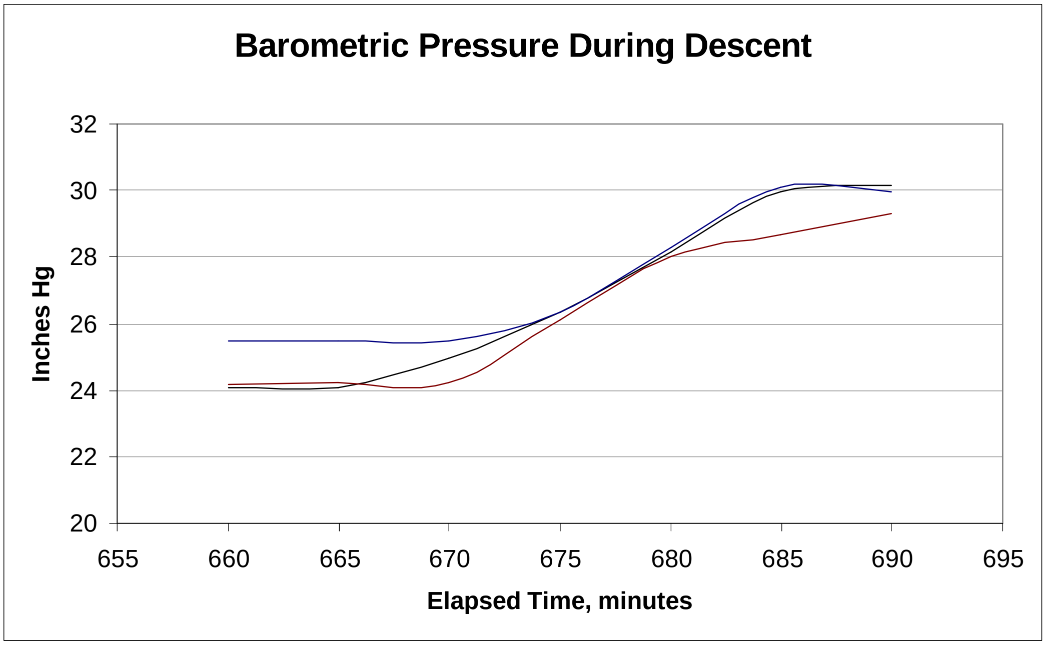

Figure 2: Barograph Traces from the Descent Phase of Commercial Aircraft Flights

data have been adjusted in time so the descent phase of the flights coincide, as shown in Figure 2.

In each case during descent, the barometric pressure rises from about 25 to 30 inches of mercury (in. Hg) in about 15 minutes. During the course of a typical, flight this fluctuation in pressure has the potential to exchange 15% of the air inside the package with the air in the surrounding environment.

For rigid packages, the pressure differential a package encounters during this pressure change can be easily approximated. Porosity measurements of common lidding materials range from 20 to 100 Gurley seconds (575 to 115 Bendtsen). For this paper Bendtsen is used to describe porosity. The Bendtsen value is the standard volume of air in cubic centimeters that flows through 100 cm^2 of material in one minute at a pressure of 1 cm water column. Assuming flow (f) is constant throughout a typical descent:

$$f = 0.15\ H / 15 = 0.01\ H \quad cc / min / cm^2$$

where H is the height of the package.

From the definition of Bendtsen:

$$\Delta P = (100\ f) / B$$

Example: Package depth 3 cm
Porosity, Bendtsen 400

$$f = 0.03\ cc / min / cm^2$$
$$\Delta P = (100 \times .03) / 400 = 0.0075 \text{ cm water column}$$

A shallow package with a highly porous lid would be representative of the low end of pressure differentials a device might experience while a deep package with low porosity would represent the high end.

Table I: Pressure differentials during aircraft descent

	Package 1	**Package 2**
Package Depth, cm	1	10
Lid Porosity, Bendtsen	600	120
Pressure during aircraft descent, cm water column	0.0017	0.083

It is important to recognize that this analysis relates only to rigid packages. Flexible packaging will easily deform due to changes in pressure. Pressure differentials experienced in flexible packages would be less than a comparable rigid package and would be a function of the stiffness of the materials and the dimensional constraints of the outer packaging.

Flow Rate through a Defect

The empirical equations used in this discussion are only approximations of the actual conditions, and the defects as considered in this discussion, are all smooth tubes or orifices. Thus, all of these results should be considered simply as guidelines.

By definition, flow in a tube is limited by the gas viscosity, while the equation for flow through an orifice is assumed to be frictionless and accounts only for the acceleration of the mass of the gas. The flow rate through a tube also depends upon whether it is laminar or turbulent. The Reynolds number for a tube is given by [8]:

$$R = \rho d v / \mu \qquad (1)$$

where ρ is the density of air, corrected for temperature and pressure:

$$\rho = 0.001293\ (273.15 / T)\ (P / 101.3)$$

and μ is the viscosity of air[9]:

$$m = m_{ref} \left(\frac{T_{ref} + T_S}{T + T_S} \right) \sqrt{\left(\frac{T^3}{T_S{}^3} \right)} \qquad (2)$$

If R < 2100, then flow in the tube is laminar, otherwise it is turbulent. In all cases of significance to package integrity, flow through a theoretical tubular defect is laminar and pressure drop through a tube can be obtained from[10]:

$$\Delta P_T = (16/R)\ (4\ l/d)\ (\rho v^2/2) \qquad (3)$$

In cases where the sides of the channel have no significant effect, such as through a large diameter orifice or in a pitot tube, the gas velocity is given by Bernoulli's Theorem[11].

$$v = \sqrt{2 \Delta P_F\ /\ r} \qquad (4)$$

The velocity as determined by Bernoulli's Theorem is the maximum rate of flow one would expect to see through a defect. Neither equation provides a satisfactory estimate of flows over the range of hole sizes of interest in medical packaging. In (3), as the tube length approaches zero, the velocity will approach infinity, while (4) makes no provision for hole size and viscous drag. To obtain some estimate of flow rate through a defect, a velocity was chosen through iteration of (3) and (4) such that:

$$\Delta P_F + \Delta P_T = \Delta P \qquad (5)$$

Table II: Predicted air velocity, cm/sec., through a hole in 0.075mm film at 0.1 cm WC

Hole diameter	**0.01mm**	**0.1mm**	**1.0mm**
Velocity through tube (3)	2.26	226	7605
Bernoulli's Theorem (4)	403	403	403
Combined equations (5)	2.26	181	400

For the purposes of a theoretical discussion, this is a reasonable estimate. The resulting estimate for flow through a hole provides good correlation with (3) for small diameter holes as well as good correlation with (4) for larger holes.

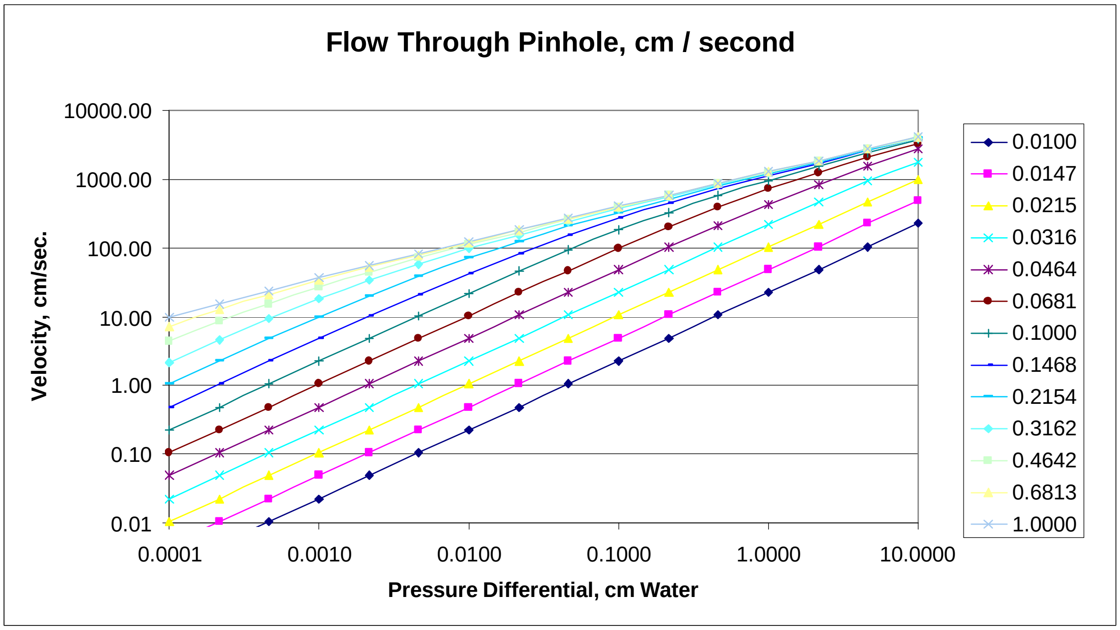

Figure 3: Theoretical Velocity of Air Through a Circular Hole in 75-µm Thick Film

<u>Particle sizes</u>

Microbial challenges come in a variety of shapes and sizes. In order to discuss the varied shapes and sizes of microbes as they relate to penetration of package defects their size will be considered to be their aerodynamic diameter, that is, the diameter of a water droplet with the same settling rate as the particle in question. For bacteria this is usually close to their physical diameter. In this presentation, because we are discussing the entire range of microbial challenges, the aerodynamic diameter of any one microbe is of little importance.

Table 3: A tabulation of the size of some microbes.

	Name	Avg. Dia	Dia / Width		Length		Eq.
			Min	Max	Min	Max	Dia.
Virus	Rhinovirus	0.023	0.018	0.028			
Virus	Hantavirus	0.06	0.05	0.07			
Virus	Varicella-zoster	0.15	0.10	0.20			
Bacteria	Parainfluenze	0.23	0.15	0.30			
Virus	Poxvirus - Vaccinia	0.23	0.20	0.25	0.25	0.30	0.08
Bacteria	Mycoplasma pneumoniae	0.23	0.15	0.30			
Bacteria	Coxiella burnetii	0.50	0.45	0.55			
Bacteria	Legionella pneumophila	0.60	0.30	0.90	2.00	2.00	0.57
Bacteria	Neisseria meningitidis	0.80	0.60	1.00			
Bacteria	Streptococcus pneumoniae	0.90	0.80	1.00			
Bacteria	Corynebacteria diphtheria	1.00	0.30	0.80	1.00	6.00	1.00
Bacteria	Acinetobacter	1.25	1.00	1.50	1.50	2.50	0.57
Mold Spore	Penicillium spp	3.30	2.80	3.80	3.00	4.00	1.00
Mold Spore	Coccidioides immitis	4.00	2.00	6.00			
Mold Spore	Rhizomucor pusillus	4.25	3.50	5.00			
Mold Spore	Cladosporium spp.	9.00	5.00	13.00			
Mold Spore	Paracoccidioides brasiliensis	18.25	6.50	30.00			

Region of Interest

The factors influencing the penetration of a particle through a defect are the pressure differential driving the flow, the defect size, and the particle size. While predictions will be made for a larger range of conditions, the conditions of greatest concern are:

Pressure differential	< 0.1 cm water column
Defect size	< 0.1 mm diameter
Particle size	< 10 µm diameter

Penetration of a Defect

To penetrate a defect, a particle must be aspirated, or drawn into the defect, and then successfully negotiate its way through the passage. These events can be treated individually and their product is the probability of a particle successfully transiting the defect.

Aspiration can be described by the equations governing aspiration into a blunt aerosol sampler[12]. These equations attempt to describe the complex flow paths illustrated in Figure 4. Comparing predicted results with actual measurements of aspiration efficiency indicates that these equations can only provide a guideline to the design of practical sampling systems. This application is well beyond the intended scope of these equations, but the results suggest that over the region of interest all particles in the air stream are drawn into the defect opening.

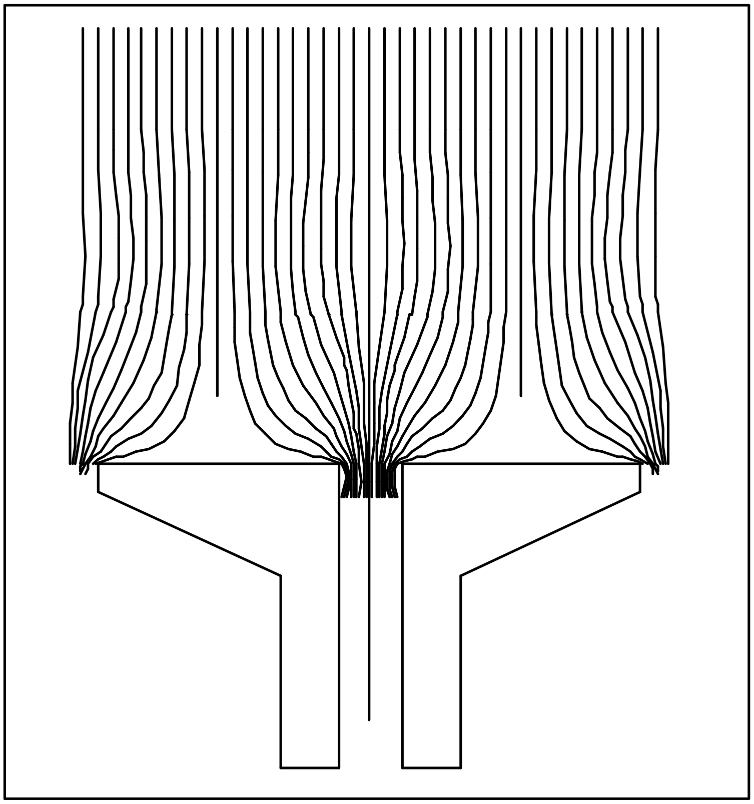

Figure 4: Airflow Around a Blunt Sampler

<u>Aspiration Fractional Efficiency</u>

$\beta = 0.752\ P\ d$

$C = 1 + (12.64 + 4.02\ e^{-0.1095\beta}) / \beta$

$r = \delta / O$

$St = d^2\ C\ U / 18\ \mu\ \delta$

$\phi = r^2\ (U_0 / U)$

$St1 = St\ \ r / \phi^{1/3}$

$\alpha_1 = 1 - \{1 /(1 + St_1 / 4)\}$

$A_1 = 1 + \alpha_1\ (1 / \phi^{1/3} - 1)$

$St_2 = St\ \phi^{1/3}$

$\alpha_2 = 1 - \{1 /(1 + 6\ St_2)\}$

$A2 = 1 + \alpha_2\ \{(r / \phi^{1/3})^2 - 1\}$

$A = A_1\ A_2$

Sedimentation due to gravity as the particle transits a horizontal defect has the potential to remove large particles from the air stream; however, because defects have a high probability of being oriented vertically it will not be considered.

Over the range of the calculations, air flow through the defect is laminar, therefore diffusion of particles through the air stream is the only means of removal during transit that will be considered. The fraction of particles penetrating a tube was calculated[13, 14, 15] for a range of hole diameters in a 75-µm (0.003") thick film for particle sizes representative of individual microbes.

Losses due to diffusion

$$B = C / (3 \pi \mu d)$$

$$D = k T B$$

$$\Psi = \pi D l / U$$

Ref. 14:

$$N/N_0 = 0.81905/e^{3.6568 \Psi} + 0.09753/e^{22.305 \Psi} + 0.0325/e^{56.961 \Psi} + 0.01544/e^{107.62 \Psi}$$

Ref. 15:

$$N/N_0 = 0.819/e^{14.62 \Psi} + 0.097/e^{89.22 \Psi} + 0.019/e^{212 \Psi}$$

The results are shown as surface plots in Figures 5 and 6. Although very small particles may be removed from the air stream in very small holes at very low pressures, in the region of interest all calculations indicate that >95% of the particles will transit the defect successfully.

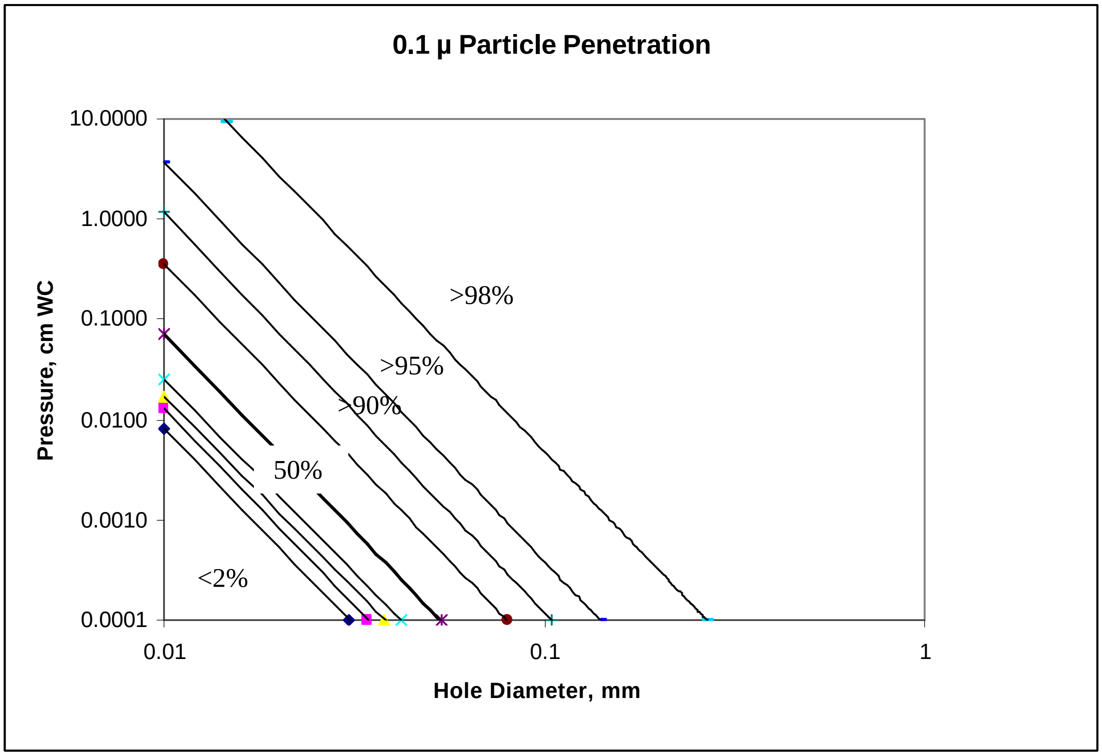

Figure 5: Predicted Fraction of 0.1 µm Particles Penetrating a Hole in 75-µm Thick Film.

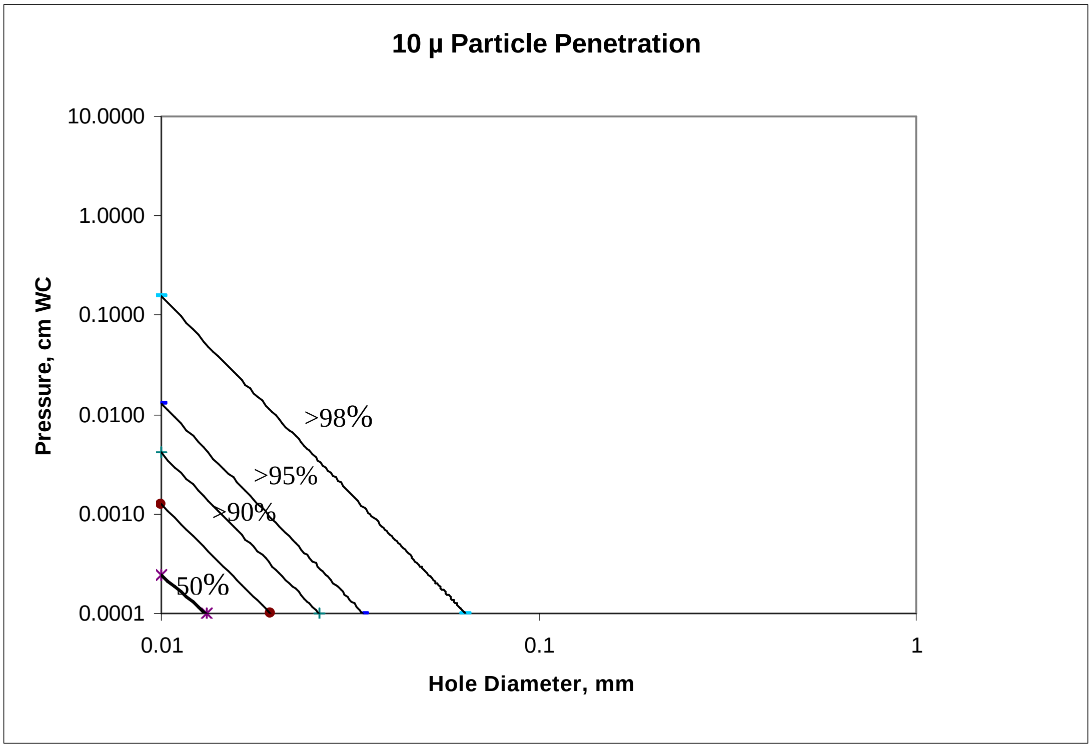

Figure 6: Predicted Fraction of 10 µm Particles Penetrating a Hole in 75-µm Thick Film.

Division of Flow

The results of the empirical analysis of the flow through a pinhole under the conditions expected during transport of a medical device indicate that essentially all aerosol particles entering a defect have the potential of being transported across the barrier. In defective packages that have a breathable component, the air flow is divided between the defect and the porous portion of the structure. Using the above equations to calculate the flow through the pinhole and through the porous package component for any given pressure, we should be able to make a prediction of the increased risk of contamination of the contents.

Comparison of Empirical Predictions with Experimental Data

A small set of data was generated two years ago using accurately sized holes drilled in 75-µm thick stainless steel. These steel shims were then glued with the drilled hole centered on a small window at the center of a 10-cm diameter disk of either coated or uncoated Tyvek® 1073B. The samples were then placed in a device designed to measure the barrier characteristics of materials at very low pressure differentials. Measurements were first taken on the samples with the hole over the open window and then with the hole sealed with some silicone rubber. Measurements were made over a range of pressures extending to the lower limits of the apparatus. The limiting factor was the presence of particles penetrating the HEPA filters delivering the sweep air. Data collection was terminated when the measured concentration was within one order of magnitude of the background concentration.

Tables 4 through 6 compare the results of the calculations with actual measured values, normalized to a challenge concentration of 50,000 p/cc and assuming 100% aspiration and no diffusion losses.

The predicted concentration took into consideration particles passing through the pinhole as well as through the barrier material. In most cases agreement between predicted and observed values is good. On coated samples with 25- and 50-µm holes, the predicted particulate penetration was much higher than was experimentally observed. The most likely cause of this disparity is the loss of particles on the surface surrounding the exit of the defect due to toroidal flow, see Figure 7.

Other possible causes of the discrepancy are the predicted flow through the defect being too high or a higher than predicted particle loss due to diffusion. Further work will be required to identify the source of the discrepancy.

Table 4: Penetration of 0.5-µm diameter particles through 125-µm holes

Sample ID	**3 U**	**102 U**	**101 C**	**14 C**
Pressure Differential, cm WC	0.10	0.10	1.50	1.02
Bendtsen	424	351	92	76
Measured Concentration, p/cc	4859	1336	2923	4325
Predicted Concentration, p/cc	2490	2950	4180	5730
Fractional Error	-0.95	1.20	0.43	0.32

Table 5: Penetration of 0.5-µm diameter particles through 50-µm holes

Sample ID	**4 U**	**6 U**	**11 C**	**12 C**
Pressure Differential, cm WC	0.20	0.10	1.01	1.02
Bendtsen	265	537	91	83
Measured Concentration, p/cc	71	211	10	12
Predicted Concentration, p/cc	170	180	395	430
Fractional Error	1.4	-0.18	37	36

Table 6: Penetration of 0.5-µm diameter particles through 25-µm holes

Sample ID	**5 U**	**7 U**	**8 C**	**9 C**
Pressure Differential, cm WC	0.20	0.30	1.03	1.03
Bendtsen	328	283	87	89
Measured Concentration, p/cc	23	1.7	1.4	1.4
Predicted Concentration, p/cc	27	11	30	29
Fractional Error	0.16	6.0	21	20

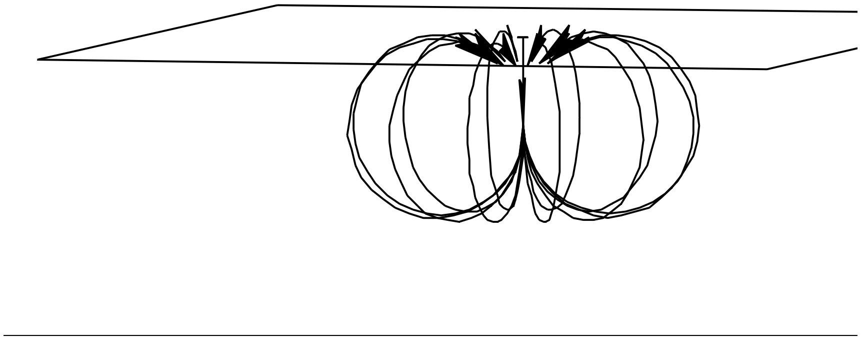

Figure 7: Toroidal flow around the exit of a defect.

Conclusion

With improved mechanistic models and data against which they can be verified, it may be possible to predict the resistance of a package to contamination based on its physical characteristics. This would allow packaging engineers to design to a level of package integrity even in the presence of a defect below the detection limit.

Acknowledgements
Dr. Paul Baron of the Center for Disease Control supplied the equations used for the calculations and their references in an Excel^{TM} spreadsheet. These equations can be downloaded in Excel^{TM} spreadsheet form from:

http://www.tsi.com/particle/coolstuf/coolstuf.htm

References

Willeke and Baron (W&B), Aerosol Measurement, Van Nostrand Reinhold 1993
Hinds, Aerosol Technology, J. Wiley and Sons, 1982

(1) Axelson, L., Cavlin,S. and Nordstrom, J.; Aseptic integrity and microhole determination of packages by electrolytic conductance measurements, Packaging Technology and Science 3:141162, 1990
(2) Chen, Harte, Lai, Pestka, and Henyoa; Assessment of package integrity using a spray cabinet technique, J. Food Protection 54(8):643-647 (1991)
(3) Gilchrist, Rhea, Dickerson and Campbell; A helium leak test for micron-sized holes in canned foods, J. Food Protection 48(10):856 – 860 (1985)
(4) Howard and Duberstein; A case of penetration of 0.2 micron rated membrane filters by bacteria, J. Parenteral Drug Assoc., 34(2):95 – 102 (1980)
(5) Lake, Graves, Lesniewski, and Anderson; Postprocessing spoilage of low-acid canned foods by mesophilic anaerobic sporeformers; J. Food Protection 48(3):221 – 226 ((1985)
(6) Lampi, R. A., Retort pouch; The development of a basic packaging concept in today's high technology era; J. Food Process Eng. 4:1 – 18 (1980)
(7) McEldowney and Fletcher; The effect of physical and microbiological factors on food container leakage; J. Applied Bacteriology, 69:190 – 205 (1990)
(8) W&B 3-1; Hinds 2-41
(9) W&B 3-10; Rader, J. Aerosol Sci. 21:2 161-168
(10) Bird, Stewart and Lightfoot. Transport Phenomena. pp182-7
(11) W&B 22-12; Lippmann in Air Sampling Instruments ACGIH 1989 p. 80
(12) Tsai and Vincent; Aspiration efficiency of a blunt sampler worn on the body; Ann. Occup. Hyg. 40(1) 93-113, 1996
(13) W&B 6-42, 43
(14) W&B 6-46 diffusion constants developed by Gormley and Kennedy
(15) Gnanasekharan and Floros; Plastic Package Integrity Testing, A Theoretical Perspective on the Minimum Leak Size for Package Integrity Evaluation; IOPP& Food Processors Inst. (1995)

Abbreviations

A	lid area
B	Particle dynamic motility
C	Slip correction factor
D	Diffusion Coefficient
H	package height (V/A)
G	Gurley seconds
O	Outside diameter of blunt sampler
P	pressure, kPa
ΔP	pressure drop
R	Reynolds number
T	Temperature, °K
T_S	Southerland constant, 101.4°K
U	Flow in tube or orifice
U_0	Flow of air stream
V	package volume

b	Bendtsen, cc air / 100 sq cm area / cm water column
f	flow, volume rate
g	acceleration due to gravity
k	Boltzmann's constant = 1.38×10^{-16} erg/°K
l	length of tube

Ψ	dimensionless diffusion parameter
δ	inlet/defect diameter

μ	viscosity, poise
μ_{ref}	reference viscosity for gas calculations: for air, μ_{ref} = 183.14 @ 296.15K and 760 mm Hg
ρ	density